Organ grafting: from the laboratory to the clinic

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In the past 40 years organ transplantation has moved from an impossibility to a well-established and major branch of surgery and medicine. Although not perfect, results of transplants of kidney, heart, liver and pancreas are good and the outcomes in patients receiving transplants of lung and intestines are improving. Patients have survived multiple organ transplants and a whole human hand and wrist has been grafted with early success. These spectacular achievements have rested on two separate approaches to problems: the first, surgical where, although there were many difficulties to be overcome – particularly in transplantation of the liver and pancreas – most surgeons agreed on surgical techniques and methods of preserving organs. The second, not yet fully solved, is the tendency to biological rejection of transplanted organs. Due to interest in transplantation and experimental clinical work in this area there has been a marked and continuing development of basic immunology.

Unfortunately, the body reacts to a potentially life-saving organ in the same way as an infecting organism. It is recognised as foreign, ‘non-self’ and a powerful mechanism is brought into action to reject the graft. Mobilisation of the immune system involves many different cells and tissues and if one prong of the immune reaction is blocked or destroyed there are effective alternative routes that can bypass the block. We have powerful agents that interfere with the immune system. Many of the drugs have a selective action on T cells, preventing their response to cytokines and their proliferation. Biologically produced antibodies, genetically engineered to a high degree of specificity directed against a single molecule, are now available, which can block the T cell receptors, prevent the T cell response to cytokines and kill lymphocytes directly.

Immunological tolerance has been the goal of clinical organ grafting since the phenomenon was first described by Billingham, Brent and Medawar in 1956. With the powerful immunosuppressive drugs and antibodies that are currently available, early results of clinical organ grafting are good, but long term functional graft survival is poor: thus only 50% of cadaveric renal allografts surviving the first year are still functioning 7.5-9.5 years later. Chronic rejection is the main cause of graft failure. The general side effects of maintenance immunosuppression are infection and malignancy, especially lymphoma and skin cancer. Specific side effects of individual drugs include nephrotoxicity (cyclosporin, tacrolimus), increased hair growth and gingival hyperplasia (cyclosporin), cushingoid changes, stunting of growth and bone necrosis (corticosteroids), and bone marrow depression (azathioprine).

Classical fetal tolerance cannot be applied clinically; however, complete ablation of the reticulo-endothelial system and re-population of the bone marrow with well matched donor bone marrow stem cells leads to tolerance in humans but also the danger of graft versus host disease. The possibility of using drugs to transform a mature immune system temporarily to the fetal state whilst an organ graft was established evolved from the experiments of Schwartz and Damashek. In 1959 they showed that the antileukaemia drug 6-mercaptopurine would prevent rabbits from producing antibodies to a protein antigen challenge.

Liver tolerance

Pigs and rats with vascularised liver allografts can become tolerant without any drug treatment. Acute rejection occurs but resolves spontaneously. Some liver allografted patients can stop maintenance immunosuppression without penalty. A short course of cyclosporin, accompanied by an infusion of donor bone marrow derived cells, can result in tolerance of experimental renal allografts in pigs. Profound T cell depletion in rhesus monkeys treated with a CD3-diphtheria immunotoxin resulted in tolerance to mismatched renal allografts.

A strict mechanistic definition of tolerance is unhelpful since an ‘operationally tolerant state’, which is 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 to the concept of happiness, something that can be achieved in different ways. We don’t always know when we are blessed with happiness but we are usually acutely aware of its loss. So tolerance, like happiness, may be complete or partial and it can be lost by a low grade, ill-understood, chronic rejection or by the precipitation of acute rejection by another stimulus - for example, viral infection or an allergic response. A minimal base line,
The non-toxic dose of maintenance immunosuppression could possibly guard against these hazards but then the state of the patient would be 'almost' or 'prope' (from the Latin) tolerance. Some patients, indeed, might not require the maintenance immunosuppression, but at present we cannot determine this by any means other than withdrawing the maintenance drug.

**The second signal**

Graft rejection is a complicated process involving many cell types, cytokines, antibodies and other factors that limit the aggression and the duration of the response in order to achieve an appropriate effect. Allograft rejection cannot occur in the absence of T cells and, to function, the T cells must be activated by two signals, namely antigen and a co-stimulatory or second signal. Blocking the second signal by an antibody can prolong allograft survival in rodents and primates. T cells receiving the first but not the second signal are abortively activated and become unresponsive (anergic) or undergo programmed cell death (apoptosis). The interacting CD40-CD154 (CD40 ligand) epitopes, respectively on the antigen presenting cells (APC) and T lymphocytes, are important components without penalty of the second signal. Ligation of this system facilitates co-stimulation of the T cell. Kirk and colleagues reported very long survival, but not tolerance of renal allografts in mismatched rhesus monkeys treated with the fusion protein (CTLA4 Ig and the monoclonal anti CD154 antibody 5C8) to block the second signal of T cell activation. Late acute rejection was reversed by repeating the treatment.

**The WOFIE hypothesis**

Patients with long surviving liver allografts may be able to stop immunosuppression without rejection of their organs. The longest survivor had a liver transplant 28 years ago and has had no immunosuppression for the last 17 years. Cautious weaning from immunosuppression of long-term liver allograft recipients would suggest that some 30% can come off all immunosuppression without penalty after five years of good function of the allograft. Stopping immunosuppression in recipients of other organ allografts usually results in their rejection. In man the liver also protects other organs from the same donor source from rejection.

We have studied the liver phenomenon for many years and a series of experiments showed that both hepatocyte parenchymal cells and bone marrow derived cells in the liver were requirements for the production of full tolerance in liver allografted rats. Neither alone would produce tolerance as tested by donor skin grafting. We also found that soluble donor class I MHC antigen is constantly produced in large quantities by an allografted liver and that the liver is a source of much of the detectable circulating class I MHC in normal people. Recently, soluble class I molecules have been shown to induce apoptosis in alloreactive cytotoxic T lymphocytes.

In all varieties of graft acceptance that do not require full dose maintenance immunosuppression, immunological engagement of donor and recipient and an early unstable period have been observed. On the basis of the hypothesis that elimination of aggressive T cell function should tip the balance in favour of a tolerant state, experiments have been performed using donor bone marrow derived cells in renal allografted, MHC mismatched pigs. An interrupted course of seven 25mg/kg intravenous doses of cyclosporin was given with a gap of two to three days without immunosuppression between the first and subsequent six doses. In kidney transplants between mismatched pigs, prolonged survival was observed in more than half the animals without any evidence of chronic rejection. This protocol with a hypothetical 'window of opportunity for immunological engagement' (WOFIE) could be suitable for clinical application. Excessive immunosuppression might prevent this engagement and the emergence of a tolerant state.

Knechtle and his colleagues have produced tolerance in a rhesus monkey model using a powerful anti-CD3 monoclonal antibody linked to a modified diphtheria immunotoxin. Three doses of the immunotoxin were given intravenously on three successive days, starting seven days prior to renal transplantation. The total dose of 2mg per kg, given over the three days, produced little toxicity and tolerance was produced in most animals receiving mismatched kidneys. Donor skin grafts were usually accepted long-term but in one experiment the skin graft precipitated rejection of the 'stable' kidney and the skin graft itself. The immunotoxin produced profound T lymphocyte depletion, which was slow to recover, and it was postulated that depletion of T lymphocytes throughout the body was necessary in addition to purging the blood.

In clinical organ grafting there is a spectrum of immunological engagement from tolerance to hyperacute rejection. Most patients with organ grafts require continuous dosage with immunosuppressive drugs. The objective would be to shift the curve to the left so that the majority of patients are 'operationally tolerant' or 'almost tolerant' and require only minimal immunosuppression.

Requirements for tolerance:
- best possible HLA match
- minimum organ ischaemia
- temporary destruction or inactivation of potential aggressive T cells.

**Prope tolerance**

Current clinical protocols of immunosuppression probably do not succeed in making the patient almost tolerant. Circulatory lymphocytes are less than 3% of the lymphocyte pool. Excessive and prolonged high dose immunosuppression may:
- prevent the host/graft immunological engagement necessary for tolerance
- cause lethal infection or lympho-proliferative disease.
The powerful immunosuppression produced by the immunotoxin used by Knechtle and colleagues had its most beneficial effect when given seven days before a kidney transplant. If the immunotoxin was given after transplantation graft survival was prolonged but rejection occurred with an antibody component. This they attributed to the lack of effect of their immunotoxin on B cells. A humanised antibody produced in Cambridge with the unique target of CD52 is a powerful depletor of T and B lymphocytes and also monocytes, but not bone marrow stem cells. In view of its effect on B cells and also the impossibility in cadaveric transplants of giving the antibody seven days before grafting, a protocol was established for giving the antibody after allografting. Thirty one recipients of renal allografts have been followed up now for between 18 and 30 months. No immunosuppression was given until the patients returned to the ward after the transplant operation. Then, with a preceding dose of intravenous hydrocortisone to control any cytokine release syndrome, the patients were given 20mg of Campath 1H intravenously. The following day a second and last dose was given. A period of 48 hours without any immunosuppression was then followed by daily cyclosporin (Neoral) to achieve a trough blood level of around 100 nanograms. No other immunosuppression was used unless there was evidence of rejection, in which case patients were treated initially with three daily doses of 1g of prednisolone. If this did not rapidly reverse the rejection, the patients were managed with dual therapy of Neoral and steroids. Infection prophylaxis was no different from standard management in our clinic. To date, there have been no serious infections and no malignancy, and no patient has lost the kidney transplant. There have been five disturbances of renal function attributed to rejection, four with a characteristic cellular infiltrate and one with a vascular type of rejection. All acute rejection responses were controlled with steroids but four of the five patients have been maintained with cyclosporin and steroids. One patient with severe heart failure prior to operation died of this condition after 11 months with a functioning kidney.

These early results are encouraging. We have called the protocol ‘prope tolerance’; or ‘almost tolerance’. Low-dose immunosuppression is the safeguard against precipitation of acute rejection by a viral infection or an allergy and also provides the clinician with a safety net, in that Neoral is a standard drug and the dose can be increased if necessary. The advantages of this protocol are complete avoidance of steroids in the majority of patients and considerable reduction in the cost of maintenance immunosuppression. The protocol is consistent with the concepts discussed at the beginning of this article but does not in any way constitute a proof of the WOFIE hypothesis. Nevertheless, one could argue that the Campath temporarily wipes out all circulating lymphocytes for approximately a month leaving the ‘slate clean’ and the slow recovery, particularly of CD4 cells, in the presence of the established graft can lead to some form of tolerance ‘mechanism(s)’. We do not yet know the degree of lymphocyte depletion in lymphocyte depots scattered throughout the body; neither do we have any data on the nature of cellular response and cytokine production of lymphocytes when they return in the circulation or on the behaviour of dendritic cells of the donor; nor do we know whether there is any change in the presentation of donor antigens. Details of these questions are now being studied, including the kinetics of the immune recovery in these patients, and a randomised trial comparing the above protocol with standard treatment is being planned.

The improved results of clinical organ grafting will increase the demand for organs, which are already not available in sufficient numbers for the patients requiring transplant operations. The supply of organs from cadavers, usually road traffic accident victims or patients who have died from cerebral haemorrhage, is unable to meet the needs of organ grafting in any country, although donor co-ordination is extremely well-developed in Spain.

Grafts from animals

The gap between patients requiring vital organs and the number of potential donors is widening every year. It is unlikely that even the most intense educational programmes and legislation favouring organ donation will ever be sufficient to provide enough donors for those in need. The only way in which the organ shortage could be drastically improved would be to use organs from animals or some kind of organ culture procedure.

Non-human primates are considered by most people to be unsuitable – man’s closest relative, the chimpanzee, is a threatened species, and more distant primates often have organs that are too small for adult humans and may harbour potentially dangerous viruses. The pig is attractive as a donor source because of the large litters, short gestation time, and methods of pig husbandry which can ensure animals that are pathogen free. Whether pig retroviruses constitute a realistic threat in an immunosuppressed human has not yet been established, but from the point of view of the science of xenografting between pig and man, considerable study and advances have recently been made. It has been known for 30 years that a pig organ can function for at least a few days in an old world monkey. However, the usual pattern of such xenografting is discordant explosive rejection with disruption of the capillary bed and massive haemorrhage occurring within a few minutes of revascularisation. The cause of this is activation of complement by naturally occurring antibodies in the primate directed against the pig. The epitope is a glycoprotein called alpha 1-gal. Attempts at overcoming the discordant rejection have included paralysing the complement system with cobra venom, and absorbing the natural antibodies with a decoy pig organ or a column with the antigen epitope; but the most attractive solution is to express the human complement control protein by genetic manipulation of a pig embryo. The transgenic pig containing decay-accelerating factor (DAF) prevents the hyperacute discordant rejection, but destructive antibodies are produced later, as well as
cellular immune mechanisms associated with endothelial activation. Attempts to overcome these xenograft reactions have required large doses of immunosuppressive agents, which would not be acceptable in clinical practice and do not give indefinite survival in experimental transgenic pig grafts to primate models.

A variety of side effects is produced by this powerful immunosuppression, including the development of lymphomas. Nevertheless, transgenic accessory, abdominally placed hearts have functioned for several months under powerful immunosuppression. More impressive has been the life-supporting function of transgenic kidneys for up to three months. In concordant baboon-to-man transplants, observations have been made which suggest that the physiologic difference of the two species could result in complications: in the longest surviving baboon-to-man liver allograft, changes in serum protein levels and the production of obstructive biliary sludge may both have been due to metabolic differences between the species. It is likely that metabolic differences between pig and primate will be much greater, and if rejection could be prevented between pig and primate, it is unlikely that the proteins produced by the pig liver would keep a human healthy. We do not know whether the physiologic behaviour of a pig's kidney or heart in the human would, in the long term, be satisfactory. A pig normally has a life-span of 10 to 15 years and an enormous growth potential in the first few months. Whether these two factors would cause serious disorder in organ xenografts is as yet unknown. It is likely that for long-term experimental organ allografts from pig to primate, some form of tolerance protocol will be necessary which could require depletion or partial destruction of the recipient immune system and possible replacement with pig stem cells. These are all unanswered questions in a scientific assault that is gathering momentum. If the transgenic approach using a single human DNA construct DAF is not sufficient, attempts will be made to introduce additional human DNA to provide other helpful proteins. The work on sheep cloning from the Roslin Institute raises the possibility of a completely different approach to introducing human DNA to the pig. The nucleus of a pig cell could perhaps be transplanted with human DNA and then cloned. There might be ethical concerns in this approach.

The assessment of what exactly is acceptable depends very much on the way in which the biological procedure is perceived. If a patient with renal failure were told that one of his blood stem cells could be manipulated by cloning techniques in a laboratory using an artificial placenta and circulation so that a kidney could be produced, this would be a perfect treatment because the organ would consist of the patient's own nuclear DNA and, after transplantation, no immunosuppressive treatment would be necessary. If, however, this step involved a surrogate animal or a surrogate human, then the whole question would probably be regarded as unethical, although when a couple decided to have another child in order to serve as a bone marrow donor for their first child sick with leukemia, this occurred without major disquiet in the community. If the child had been conceived for the purpose of providing a kidney for donation, the ethical perception would have been very different, and if it had been to provide a vital unpaired organ undoubtedly this would have been regarded as totally unacceptable. It is perhaps sensible at least to address these hypothetical questions while they are still hypothetical. There can be no doubt that the pressure to increase the donor organ supply is extremely strong and many clinicians involved in organ transplantation do have worries and experience disquiet both concerning the removal of an organ from a volunteer donor who is perfectly healthy, and the circumstances that are involved in removing organs from brainstem dead cadavers.

In the past 40 years transplantation has blossomed from a crazy and probably impossible idea to a form of treatment that is accepted and wanted for all patients with chronic renal failure and severe diseases of other vital organs. There has been a proliferation of immunosuppressive drugs following the introduction of cyclosporin in the 1980s, but we are still learning how best to use them. We expect cadaveric renal transplants to have an 80–90% one-year survival but this figure falls to around 50% at 10 years. This highlights the main obstacles that need to be overcome for further improvement: namely chronic rejection, chronic nephrotoxicity, and other side effects of immunosuppression, particularly neoplasia and the tendency for atherosclerotic cardiovascular disease to progress. The goal remains before us.

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**Domiciliary oxygen therapy services**

**CLINICAL GUIDELINES AND ADVICE FOR PRESCRIBERS**

**Report of a working party of the Royal College of Physicians**

The provision of oxygen cylinders and oxygen concentrators costs the NHS approximately £30 million annually. Although criteria for the use of long-term oxygen in chronic obstructive pulmonary disease are well established, a number of studies have revealed variability in prescribing habits, poor adherence to present guidelines and lack of organised follow-up and monitoring arrangements. The Department of Health therefore requested that a multidisciplinary working party, under the auspices of the Royal College of Physicians should provide new guidance for the use of domiciliary oxygen. Indications are given in the report for the provision of oxygen therapy for adult patients with chronic respiratory disease and also in paediatrics, cardiology and palliative medicine. The organisation of home oxygen services is discussed in detail and proposals are made for improved methods of prescribing and for patient education. Recommendations are accompanied by levels of evidence and the report includes an extensive bibliography to support its findings. The report has been prepared for those involved in the care and assessment of patients with respiratory and cardiac disease who require domiciliary oxygen. In particular, it is targeted at general practitioners and general physicians, geriatricians, palliative care physicians and cardiologists. Nurses, physiotherapists, occupational therapists and pharmacists will also find much valuable guidance in the report. The guidance on services required for assessment, prescribing and follow-up for patients on home oxygen therapy will be required by health service commissioners and primary care groups. Patients who use oxygen services will also find much useful information in this report.

**Foreword by KGMM Alberti PRCP**

1. Definitions and Abbreviations  
2. Introduction  
3. Recommendations  
4. Indications and assessments for oxygen therapy  
5. Technology for provision of home oxygen therapy  
6. Organisation of home oxygen services  
7. Paediatric domiciliary oxygen therapy  
8. Travel for patients on domiciliary oxygen therapy  
9. Appendix: Domiciliary oxygen record forms

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