Commentary

The Role of Research in Transplantation

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Abstract

In the past 50 years, organ transplantation has developed from an improbable laboratory exercise to a major therapeutic success. The surgical problems of organ grafting have, for the most part, been solved. Rejection of grafts is now partially understood and usually controllable by powerful immunosuppressive drugs. A steady improvement in patient outcome, especially following the introduction of cyclosporin as an immunosuppressive agent has resulted in a worldwide shortage of organs for transplantation. This has provoked serious ethical dilemmas in every country. These matters are summarised in the following text.

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Introduction

Organ and bone marrow transplantation have been extraordinary success stories in medicine during the past 50 years. A large and important clinical service has been developed in both these subjects and there has been a staggering improvement in the overall results of transplantation of patients who previously were offered no treatment for a variety of fatal diseases. Long-term good functional survival of the transplant has been observed in an increasing number of patients; some are more than 40 years after the transplant procedure. Transplantation would not exist in the clinic without a powerful backing of basic and transitional research, progress being manifest in a partial understanding of the rejection process, the immune system and how to circumvent rejection. In the 1950s, there appeared to be 2 problems, namely surgery and biology. It was not clear until the first identical twin kidney transplant in 1954 that the human kidney could withstand the procedure which involved removal from 1 individual, a period of ischaemia during the implantation and the actual surgery in the recipient to provide new arterial and venous access and uretic drainage.[1] There were sporadic attempts at kidney transplantation during the 20th century and important observations were made by Alexis Carrel who had introduced a method of joining blood vessels together and had used this in experimental kidney grafting in animals. Carrel himself pointed out that "autografts" could be successful with long-term function but grafts between unrelated individuals, later called allografts, might function initially but were doomed to failure by mechanisms not understood then. Gradually surgical techniques were developed for experimental transplantation of the liver, heart, lungs, pancreas and spleen. The early literature was dominated by discussions of different technical procedures and it became clear that in all the organs mentioned above, depriving the tissue of a blood supply should be reduced to a minimum. Cooling gave additional time so that the organs remained alive, just as in the principal of the refrigerator, and the main blood vessels required full restoration of blood flow but the lymphatic system and autonomic nerves were not vital to initial function and regeneration was to be expected with time.

The controversies of the early days regarding technique have now largely subsided except in new areas of transplantation, for example, the limbs and face where nerves, tendons and bone must also be considered. One of the most interesting observations in hand transplants have been in neuro-physiology, the cortical areas involved in hand sensation and movement atrophy after loss of the hands by accident or amputation. Following successful hand transplantation, these critical areas regenerate, demonstrating plasticity in the brain not previously anticipated.

The heart/lung machine maintaining life during cardiac surgery was essential to the development of heart and lung transplantation. Advances in anaesthesia and metabolic

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control during the operation were necessary for successful liver grafting.

All these surgical considerations were studied and successfully overcome in the laboratories of surgical departments by surgical scientists, but they have always required collaboration with more basic scientists in trying to understand and control the immune response.

Rejection

Skin grafting from one part of an individual to another site has been practised for hundreds of years and surgical techniques have developed so that treatments could be used in burns and other lesions where there was skin loss. Grafts from another person, however, invariably failed after appearing to look satisfactory for about a week to 10 days. An exception was grafts between identical twins called "isografts". The seminal studies by the biologist, Peter Medawar, and his surgical colleague, Thomas Gibson,[2] showed that the nature of graft rejection had the properties of an immune response and a second graft from the same donor to the same recipient after the first had been rejected was much more rapidly despatched. The histology of the first reaction showed infiltration of the graft with lymphocytes whereas in so-called "second-set" rejection, lymphocytes were far less prominent and Gibson and Medawar showed that a specific immunity had been established and postulated that antibodies could well be involved. Thus, rejection had characteristics similar to immunity that follows an infection. There is an "immunological memory" specific for the donor in question. With hindsight, it could be postulated that in embryonic development there must be some mechanisms to ensure that cells of the reticulo-endothelial system, wherein lay immune reactivity, would require some means of recognising and reacting against foreign antigens, whilst accepting proteins of the natural environment of the organism. Many years later, it was found that this "education" of lymphocytes occurs in the thymus which eliminates potentially dangerous lymphocytes and permits the survival of protective cells that are harmless to the individual. Of course when this process goes wrong, the stage is set for an autoimmune reaction.[3,4]

Medawar and his colleagues set out to differentiate between identical and non-identical cattle twins, expecting the non-identical cattle twins to reject each other's grafts, but to the surprise of the scientists, they accepted them for long periods and this was the first clear demonstration of "natural immunological tolerance".[5] The hypothesis arising from these experiments was that the immune system before it was developed, would accept any protein antigens present in its local environment as its own. The placenta of cattle twins is unusual in that blood freely circulates between the twins in the placenta *in utero*. Proof of this hypothesis rested on the demonstration of actively acquired immunological tolerance, which Billingham, Brent and Medawar reported in mice. They injected cells from one highly inbred strain into the foetus or neonate of another inbred strain. Subsequently, grafts from the donor strain were accepted, usually for long periods but not in all cases indefinitely.[6] An unexpected by-product of these experiments was some of the animals became emaciated and looked like runts. The so-called runt or secondary disease was shown to be due to a "graft-versus-host" reaction where the transplanted cells reacted against the host.[7]

In addition to the so-called acquired immune response, all vertebrates have retained a more primitive innate immune mechanism in which white blood cells recognise and destroy any foreign antigenic protein immediately.

Already this simplified story, summarised above, was becoming complex but since it was possible to surgically transplant not only skin but also organs, there was a strong stimulus for research workers to try to overcome the immune response in animals with a mature immune system with a view to eventual clinical application. It was known that the immune system was susceptible to high doses of x-irradiation. The doses required were lethal, destroying all rapidly dividing cells particularly in the skin and the intestine as well as the bone marrow. It was postulated that a bone marrow transplant might rescue an individual subjected to high dose x-irradiation and this proved to be the case. Bone marrow transplants do not require a surgical procedure. The progenitor cells in the bone marrow automatically "home" to appropriate sites in bone when injected intravenously. Bone marrow aspirated from the experimental animal before irradiation was found to be capable of rescuing the animal if injected after irradiation. Allografting soon demonstrated certain rules that fitted in with the concept of "tissue types" additional to and distinct from red blood cell groups. If the bone marrow came from an identical twin or a sibling of identical tissue type, then the grafts were usually successful although in the latter case, a graft against host disease might occur. With grafts from donors unmatched for tissue types, rejection of the marrow occurred. Bone marrow grafting can be life-saving for non-malignant bone marrow diseases, for example, aplastic anaemia, and also sometimes in the treatment of leukaemias. In leukaemia cases, the graft against host disease seemed to be beneficial, in having a selective, enhanced immune reaction against the leukaemic cells.

In organ transplantation, the clarity of rules for graft acceptance was less clear. Irradiation alone was not effective in permitting kidney graft survival except in 2 unusual cases where the donor and recipient were non-identical but tissue matched twins.[8,9] For several years, patients with kidney

disease were subjected to irradiation which either killed them or did not prevent kidney graft rejection.

With this dismal background, it seemed reasonable to investigate other agents used to treat leukaemia that might have a better therapeutic index than x-irradiation. The anti-leukaemic drug, 6-mercaptapurine (6-MP), was shown by Schwartz and Damashek to prevent rabbits from producing antibodies, not only whilst the drug was given, but the effect was long lasting.[10] This seemed to be a useful starting point for chemical immunosuppression and 6-MP was found to prolong kidney graft survival in dogs, sometimes for long periods.[11,12] An analogue of 6-mercaptapurine, aziothiaprine, was slightly superior to 6-mercaptapurine.[13] This agent was the first clinical application of research into chemical immunosuppression that actually benefitted patients, especially when corticosteroids were added to the aziothiaprine.[14]

During the next 20 years, many different agents were investigated. The serum of animals injected with human lymphocytes contained many antibodies, which could be immunosuppressive. Initially, these polyclonal serum antibodies were difficult to produce as effective non-toxic preparations. The demonstration of monoclonal antibodies with a single molecular target by Kohler and Milstein[15] led to more predictable products and various target epitopes for monoclonal antibodies were investigated. There are some very powerful monoclonal antibodies available for clinical use.

The cyclic fungal peptide cyclosporin was reported by Borel from the Sandoz laboratories to have immunosuppressive properties *in vitro* to prolong skin-graft survival in mice but was difficult to work, being almost totally insoluble in aqueous solutions.[16] When dissolved in oil, encouraging results were obtained with cyclosporine in rodents with heteropropic heart grafts, dogs with kidney grafts and pigs with orthotopic heart grafts.[17-19] Cyclosporine was used in the clinic for the treatment of recipients with kidney grafts. Once the correct dosages had been established, cyclosporine improved the 1-year functional survival of kidney grafts to 80%, having been previously around 50% with aziothioprine and steroids.[20]

The stage was now set for transplanting other vital organs, namely the individual heart, liver and lungs. Since each of the immunosuppressive agents studied had side effects, a strategy was adopted of giving several drugs in low doses, hopefully to add to their immunosuppressive activity but with a reduction in their individual side effects. Such regimens were established 20 years ago and have continued to be refined, while new agents from the laboratory have been investigated. A powerful agent discovered in Japan was FK506 or tacrolimus, which has a similar mode of action to cyclosporine but with different side effects. Rapamycin (sirolimus), an agent with a similar molecular structure to tacrolimus but which has a different mode of action, was found to be a useful addition to immuno-suppressive therapy.

An interesting observation from many years ago was that different organs had a varying susceptibility to rejection, the skin and intestine being particularly vulnerable, and the liver being relatively privileged. In fact, liver transplantation in pigs and rodents could sometimes survive indefinitely without any immunosuppressive therapy.[21]

This phenomenon of liver tolerance was demonstrated rather dramatically by some patients of Thomas Starzl, who stopped taking their immunosuppression without telling their doctor. Some 30 of these patients have survived long-term, a few for more than 20 years, without any maintenance immunosuppression.[22] Tolerance involves a very critical balance between the immune potential of the recipient, the antigenicity of the donor, the initial immunosuppressive regimen, and the organ involved, together with the tissue typing disparity of the donor and recipient.

There has been a tendency for transplant doctors to add new agents to the clinical protocol, often resulting in over-immunosuppression, toxic side effects and probably interfering with natural switch-off immune mechanisms, which may well be involved in the establishment of tolerance. There is now a swing towards minimal immunosuppression consistent with functional graft survival. This has been helped by powerful induction therapy, in particular using the monoclonal antibody, Campath 1H, which causes depletion of circulating human lymphocytes, particularly T cells. Half the normal dose of 1 drug, instead of the full dose of 3 agents has resulted in good functional survival of grafts for up to 8 years, with many patients not requiring steroids at any time and enjoying remarkably good quality of life.[23,24] This regimen is much cheaper and is an advantage for those endeavouring to establish organ transplantation in developing countries.

The Future

The current problems in organ transplantation are largely due to the success of the procedure, namely an increasing disparity between those needing a transplant and the availability of organs. The ethical aspects of this dilemma are outside the scope of this article, but obviously an improvement in the long-term results of organ transplantation and a reduction in the cost, particularly of maintenance immunosuppression, would go some way to relieving the ethical tensions. On-going research concentrates on the causes, prevention and treatment of chronic rejection. This is an insidious process which affects mainly the small arteries of the graft, resulting in re-

duplication of the intima, macrophage infiltration and eventually, occlusion and ischaemia of tissue supplied by the artery. Special manifestations of this process are a major cause of failure in heart transplantation and the bronchiolitius obliterans of lung transplants. The role of antibodies in chronic rejection has received much attention and is probably important.

Research also continues in minimising immunosuppression with the possibility of producing operational tolerance in some patients. In a population of organ graft recipients, we do not have the means of predicting which patients can be safely weaned off immunosuppression and the "trial and error" approach can lead to rebound rejection and permanent damage or even loss of the organ.

New ways are being sought to prevent activation of lymphocytes by interfering with the second signal between lymphocytes and antigen-presenting cells to prevent the initiation of the immune response. In the laboratory, approaches of this nature have been remarkably successful, but so far they have not been realised in the clinic. It would appear that the human species has alternative pathways for the rejection of grafts, in addition to innate and acquired immunity.

Other important areas for research are the prevention of recurrent disease, for example, glomerular nephritis in renal transplant recipients, tumours and viral hepatitis in liver transplant patients and aggravated coronary artery disease in heart grafts. All currently used immunosuppressant drugs have side effects and therefore none is definitive or ideal, so there is room for the investigation of new immunosuppressant agents.

Vascularised pancreas transplantation has now become very successful, particularly in patients receiving induction therapy and minimal maintenance immunosuppression. Nevertheless for many years, an attractive alternative has been the concept of isolating the islets Langerhan from the pancreas and grafting them into the liver. Excellent early results were achieved by workers in Edmonton using a "cocktail" of immunosuppressive agents to avoid, as far as possible, toxicity to the islets and selecting patients who had not yet developed severe secondary complications of diabetes, but were in danger of unaware hypoglycaemic unconscious attacks, which can be fatal. This programme, spearheaded by James Shapiro, saw 80% of the patients no longer needing exogenous insulin.[25] However, there was attrition of graft function so that by 2 years, 70% of the patients did not require insulin injections, but by 5 years, most of the patients needed extra insulin, although many had been cured of the unaware dangerous hypoglycaemic attacks. The reasons for the disappointing late results are probably multiple. The following are possible contributors: inability of progenitor cells to replace dead beta cells, toxicity of immunosuppressive drugs and recurrence of the autoimmune disease which caused the type 1 diabetes. So for the moment, the vascularised pancreas is the best option, but this and islet transplantation both have the inherent defect of requiring cadaveric donors. There has therefore been extensive interest in research to try and overcome xenograft rejection. Despite a huge literature and vast sums expended, to date there has been no long-term success of any xenograft in the clinic. An alternative approach is gene and/or stem cell therapy, which could in some diseases, for example diabetes, overcome the need for cadaveric tissues and instead of only a few patients being able to benefit. Gene therapy in theory could be available to all patients with appropriate indications. Sadly, the path of gene therapy after initial "hype" has been disappointing with a variety of problems still to be overcome. It will be necessary to obtain sufficient gene transfection to be effective to prevent gene silencing, and to control storage and release of the protein in question, for example insulin. There are potential dangers of oncogenesis and cell damage of viral vectors which are currently necessary to obtain sufficient transfection. A recent encouraging approach has been reported by Ann Simpson's group in Sydney, using a lenti viral vector containing the human insulin gene infused with the portal vein of a temporarily ischaemic rat liver.[26]

There can be no doubt that future advances in transplantation will depend, as in the past, on a base of intelligent research and with co-operation between different disciplines.

Conclusion

From the above review, it is clear that there would be no organ transplantation without research, both basic and translational, from many areas including medicine, surgery, biology, pharmacology and molecular biology.

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