Section 1 General

Chapter 1

Historical perspectives

John Dunning and Sir Roy Calne

Key points

- Successful techniques for vascular anastomoses developed at the end of the nineteenth century made the transplantation of internal organs possible.
- The first successful human allograft, a corneal transplant, was performed in 1905.
- The recognition that the body's reactions to foreign tissue led to the failure of allograft transplantation gave rise to the new discipline of immunology.
- The discovery that cyclosporine, a metabolite from the fungus *Tolypocladium inflatum*, is 300 times more active against the proliferation of splenic lymphocytes than against other cell lines changed the face of transplantation.
- As transplantation has become more successful in terms of survival, quality of life, and cost benefit, the demand for donor organs has increased so that it is now greater than supply.

Transplantation of organs represents the pinnacle of medical achievement in so many different ways. It epitomizes the multi-disciplinary team approach to patient care. It has a foundation in refined surgical technique, supported by an understanding of complex immunological events, and requires a complex approach to pretransplant assessment and postoperative care of multiple organ systems. Yet in some respects it also represents a failure: the inability to repair diseased organs such that the only way forward is to cast aside the worn out tissue!

The idea of organ and tissue transplantation is not new, and reference to it may be found in the ancient literature of China and India. The first description of a skin transplant is contained in the Sushrutta manuscripts dating from around 450 BC. The technique described found use in Europe during the Middle Ages in the hands of the Italian surgeon Gaspare Tagliacozzi. He used it for the reconstruction of damaged noses, frequently a result of syphilitic injury, using a skin flap from the forearm. At the time he wrote that "the singular nature of the individual entirely dissuades us from attempting this work on another person." Perhaps he had already attempted the repair using allogeneic donors (transplantation between genetically disparate individuals) prior to his successful autograft (transplant of tissue in the same individual). Although the technique was new to the people of the time, the concept of tissue transplantation was well established among Europeans following the legend of a total leg transplant by Saints Damon and Cosmos illustrated by artists such as Fra Angelico and sculpted by Donatello. Such legendary optimism was not rewarded clinically until much later, but it is certain that interest in skin grafting was revived due to the substantial need for treatment of the gross leg ulcers prevalent in the nineteenth century as a result of injury from syphilis, nutritional deficiency, and burns. Great advances were made with the observations of the French Physiologist Paul Bert, who recognized the importance of graft neovascularization and described the success of autografting in comparison with the failures of allografting.

It was the ophthalmic surgeons who really led the way to successful allografting with the transplantation of corneal grafts. Samuel Bigger reported what was probably the first successful full-thickness corneal allograft when he performed an operation on a blind pet gazelle while he was a prisoner in Egypt in 1835. He replaced the cornea, apparently with good results.

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Attempts to reproduce this success continued through the latter part of the nineteenth century, and with technical improvements and increasing frequency of trials, the results with animal corneal grafts improved steadily. Finally, in 1905, the first successful human corneal allograft was performed. Although therapeutic transplantation of the cornea became firmly established as part of ophthalmic practice from this time, there was no theoretical explanation why corneal grafting should be successful whereas the grafting of other organs and tissues was not, nor of the observation that from time to time corneal grafts were rejected.

It was not until Alexis Carrel and Mathieu Jaboulay developed successful techniques for vascular anastomoses at the end of the nineteenth century that the transplantation of internal organs became possible. Many different animal models were used with attempts to transplant almost every organ, but the kidney was the first organ to which this technique was repeatedly applied.

Carrel remained a prominent contributor to the field of transplant surgery throughout the early 1900s, moving from France to the United States, where his collaboration with Guthrie led to significant contributions to vascular surgery with the development of techniques for venous patching of arteries and the use of cold storage to protect tissue for reimplantation up to 20 hours from its procurement. The result of their labors was a series of 35 papers describing their experimental achievements in a wide variety of animal models for transplantation. However, it was not until 1908 that survival became extended when Carrel performed a kidney transplant in a dog with survival of the graft for several years. With the survival of grafts beyond a few hours, the opportunity to study tissue histologically emerged, and by 1905, parenchymal infiltration by "round cells" and arterial lesions were recognized.

Of course human donors were not available at this time, and all organs transplanted were obtained from animals so that a mixture of pig, goat, monkey, and sheep *xenografts* (transplantation between species) were undertaken in human patients with acute renal failure. Although none of these attempts were successful, the last attempt by Neuhof in 1923 was particularly encouraging, with the recipient surviving for 9 days. It demonstrated clearly that thrombosis and hemorrhage from vascular anastomoses was not inevitable. Although most attempts to perform organ transplantation were made in animals, Mathieu Jaboulay attempted the technique in man, and in 1906 he reported his observations in Lyon Medical. His attempts used a pig kidney in one patient and a goat's kidney in a second, with the organs implanted in the cubital fossa and anastomosed to the humeral artery and cephalic vein. Ultimately both attempts failed as a result of vascular thrombosis, but the kidneys did start to diurese initially.

It quickly became apparent that whereas autografts generally succeeded, allografts and xenografts mostly failed. Although the technical problems of the operation had largely been sorted out, it was clear that "from a biological standpoint . . . the interactions of the host and of the new organ are practically unknown." The increasing understanding that the resistance to foreign grafts was caused by systemic factors led to the repeated suggestion that an immune response of the "anaphylactoid type" was somehow responsible for graft rejection. It was recognized that research had now to be directed toward understanding the body's reactions to foreign tissue, and so from experimental transplantation in the early part of the century, the two new disciplines of vascular surgery and immunology emerged.

Other landmarks were reached throughout the early years of the twentieth century, with growing understanding of skin grafts used to treat burns, and with Voronoy transplanting the first cadaveric human kidney in 1933. His recipient was a 26-year-old woman who had attempted suicide by swallowing sublimed mercury. This led to uremic coma. The kidney was procured from a 60-year-old man who died following a fracture of the base of the skull. The operation was performed on April 3, 1933, with the renal vessels anastomosed using Carrel's technique to the femoral vessels and the kidney placed in a subcutaneous pouch, with externalization of the ureter. Local anesthetic was used. The donor was known to be blood group B, and the recipient blood group O. The grafted kidney did diurese for a while, but unfortunately the patient died 2 days later.

Despite the demonstration of second-set skin graft rejection in man as early as 1924 and the successful exchange of skin between identical twins in 1927, no useful generalizations were made to further elucidate the immunological mechanisms involved. The practice of corneal grafting continued, but it seemed to be accepted that the transplantation of other tissues and organs was impractical, and there was a lull in activity among surgeons for the next 20 years, with further

interruptions to the field brought about by the Second World War.

The area of skin grafting became of greater importance for the treatment of war burns and other injuries, and the death from kidney disease also provided impetus to focus once more on kidney transplantation. Short-term success in the late 1940s was reported by a number of individuals, including Voronoy, and David Hume working in Boston. Both transplanted kidneys into patients with uremic coma that diuresed for a number of days, before stopping and being removed again. The technique was not seen as replacement therapy but a method of stimulating a recovery reflex in the native diseased kidney. However, as the immunological basis of rejection became established, scientific interest in organ transplantation waned until effective immunosuppressive regimens were found.

Abdominal organ transplantation

Transplantation of abdominal organs has been a long-term success story, with patients surviving 40 years with excellent function in their original grafted organs. The success of clinical allograft transplantation began with transplantation of kidneys between identical twins by Murray and colleagues at Peter Bent Brigham Hospital in Boston in 1956. This was an outstanding achievement and demonstrated clearly that the kidney would withstand the trauma of removal, periods of ischemia, and then the procedure of transplantation into another individual of the same species. The fact that identical twins would not be able to reject skin grafts and the experimental auto-transplantation of the kidney in the dog enabled the group in Boston to proceed with the clinical operation with reasonable optimism. Unfortunately, a twin donor would not be available for most patients dying of kidney failure, and the immunological barrier between individuals proved to be an enormous biological problem.

For more than a decade, clinical kidney transplantation was the only form of organ grafting that was seriously studied and yielded some success. The identical twin experience was reproduced, and conditioning of the recipient with total-body irradiation was applied to kidney grafting between donor and recipient who were not twins. This was based mainly on experimental work with bone marrow transplantation; however, in the clinic the results were disastrous, except in two cases of kidney grafting between non-identical twins. Patients subjected to total-body irradiation frequently succumbed to infection, aplasia, and cancer.

The introduction of chemotherapy to supplement irradiation and allow dose reduction improved the outcomes further, and in 1960, William Goodwin introduced methotrexate and cyclophosphamide to the field of living related transplantation and treated an episode of rejection with prednisolone. Then, in London in the mid 1950s, the prolongation of survival of renal allografts in dogs by the anti-leukemia drug 6-mercaptopurine (6-MP) heralded clinical immunosuppression and azathioprine (AZA), a derivative of 6-MP, was found to be slightly better experimentally. Although 6-MP was used briefly with irradiation, it was rapidly abandoned because of significant toxicity. The use of AZA in clinical kidney transplantation was originally disappointing, but when corticosteroids were added, this immunosuppressive regimen resulted in some long-term clinical renal allograft successes from the early 1960s.

Further understanding of transplant immunology was gained with insights into the human leukocyte antigen (HLA) system and histocompatibility. Crossmatch techniques became established through the 1960s, and understanding of the "transfusion effect" was also gained (Opelz and Terasaki), whereby previous transfusion appeared to confer protection for the transplanted organ.

In the 1960s, experimental transplantation of the liver, pancreas, intestines, and heart led to a clarification of the technical requirements involved, and in 1963, Starzl in Denver carried out the first clinical liver transplant. Unfortunately, the results of this clinical series were dismal, and Starzl self-imposed a moratorium until 1967, when he resumed clinical liver transplantation, having in the meantime improved the surgical technique and the assessment of graft function and prevention of rejection. The first orthotopic liver transplant in Europe was performed in Cambridge by Calne in 1968. For nearly 10 years, Denver and Cambridge were the only two centers with regular programs of clinical liver transplantation. There were a few outstandingly good results, but many disappointments. Patients were referred for operation too late, and anti-rejection therapy was still in the process of development using modified regimens of AZA, steroids, and polyclonal anti-lymphocyte serum. In addition to rejection, sepsis, biliary, and vascular complications and recurrence of the patient's own disease often resulted in failure. During this uncertain

and disappointing phase of development, the vascularized pancreas was also transplanted and shown to be capable of curing diabetes in a few patients. However, many patients suffered from complications of leakage of pancreatic enzymes, causing inflammation and fatal sepsis.

A watershed in organ transplantation was the discovery of the immunosuppressive properties of cyclosporine (CyA), a metabolite from the fungus *Tolypocladium inflatum*, by Jean Borel working in the Sandoz laboratories. CyA was 300 times more active against the proliferation of splenic lymphocytes than against other cell lines. Experimental and clinical application of CyA transformed the attitude of previously sceptical clinicians to organ transplantation. Calne's paper published in *The Lancet* in 1979 described its use in 32 kidney transplants, 2 liver transplants, and 2 pancreatic transplants and showed improved 1-year functional survival of kidney transplants from below 50% to approximately 80%. It was introduced to clinical immunosuppressive regimens worldwide in 1982 and radically improved the survival of heart, kidney, liver, and pancreas recipients. About 10 centers had soldiered on in the pre-CyA era, but after the introduction of CyA, there were soon more than 1000 centers. The improved results led to an expanding mismatch of numbers of available donors to potential recipients seeking a life-saving organ graft.

Unfortunately, the nephrotoxic side effects of CyA led to late renal failure in many cases. Hopes that there might be a dosage window in which rejection could be controlled and side effects avoided were only realized in a minority of cases. However, the concept was established of combining immunosuppressive drugs with the objective of obtaining added immunosuppressive effect but reducing the individual side effects. Thus AZA, CyA, and steroids became a standard immunosuppressant regimen.

The liver proved to be less susceptible to rejection than other organs. This had been anticipated by experiments in pigs and rats. In an important "patient-led clinical study," a group of patients from Denver stopped taking their maintenance immunosuppression without telling their doctors. Although lack of compliance is a common cause of organ graft failure due to rejection, a surprising number of young patients with liver transplants did well long-term. A number of patients, in whom immunosuppression was stopped for medical indications, usually infection, also did not require renewal of their immunosuppressive regimen of drugs. Confidence in the surgery and immunosuppression gradually increased.

A variety of complicated organ graft procedures were reported, including small bowel on its own (1988) and in combination with liver and other organ grafts. The first combined heart, lung, and liver transplant was performed by Wallwork and Calne in 1987 at Papworth (Cambridge, United Kingdom), with survival of the patient for more than 10 years.

There is now a move toward minimization of immunosuppression and tolerance. Alemtuzumab (Campath), an extremely powerful anti-lymphocyte antibody developed in Cambridge by Waldmann and colleagues, has induced "*prope* or almost tolerance" when used as an induction agent followed by maintenance immunosuppression with half-dose CyA, rather than a full dose of three drugs. Of the original series of kidney transplantation patients treated in Cambridge, 80% have never received steroids, and their quality of life has been excellent after more than 10 years of follow-up. This immunosuppressive regimen has reduced complications of anastomotic leakage in pancreas transplants, with encouraging results.

Pancreas grafting can never be a treatment for all diabetics, but when transplanted together with a kidney in patients with diabetic renal failure, pancreas transplantation has produced excellent long-term results. A move toward islet transplantation to avoid the major operation has had some early encouraging results. This is a field in which stem cell and/or gene therapy may well lead to fruitful developments in the future.

Cardiothoracic transplantation

While the field of kidney transplantation research and experimentation moved rapidly into the clinical arena, progress was not so rapid for the transplantation of other organs. The first heart transplant described in the literature was performed in 1905 by Carrel and Guthrie. The heart, transplanted from one dog into a heterotopic position in the neck of another dog, continued to beat for 2 hours. This model demonstrated that it was possible to transplant a heart with all four chambers pumping blood. More importantly, it demonstrated that the heart could be removed from its blood supply and sutured into the circulation of a second animal and still recover its normal organized

contractile pattern. This brought into focus the concept of "preservation" of the heart during its ischemic period.

Further reference to transplantation of the mammalian heart was made in 1933 by Mann and colleagues at the Mayo clinic, who were seeking a denervated heart model. They made contributions to the area of preservation, advising that ventricular distension and coronary air embolism should be avoided; made observations on the general behavior of the transplanted heart; and made the first observations on the phenomenon of cardiac allograft rejection, noting that "histologically the heart was completely infiltrated with lymphocytes, large mononuclears and polymorphonuclears." They concluded that "the failure of the heart is not due to the technique of transplantation but to some biologic factor. . . ."

Interest in cardiac transplantation waned until 1951, when workers at the Chicago Medical School reported their experience with a slightly modified Mann preparation. They were interested in the possibility of transplanting organs as a treatment modality for end-stage disease, but their experiments, although elaborate, were disappointing, with a maximum survival of only 48 hours. It was apparent to them that "the greatest deterrent to long survival of the heart is the biologic problem of tissue specificity" and concluded that "a transplanted heart . . . must be considered, at present, a fantastic dream, and does not fall within the scope of the present considerations." The Mann preparation continued to be used by various investigators to evaluate the transplanted heart, and Downie, working at the Ontario Veterinary College, reported excellent results, which he attributed to the use of penicillin and appropriate commercial suture material. Demikhov published results in 1962 in which an intrathoracic heterotopic heart continued to beat for 32 days. The long survival of this graft strengthened his belief that failure of transplanted organs was not due to immunological factors, but to simple technical problems.

The successful intrathoracic transplantation of the heart without interrupting the circulation led to the idea that a cardiac allograft might be able to assume some of the normal circulatory load. Demikhov led the way, performing 22 such auxiliary heart transplants between 1951 and 1955. The donor heart was implanted, and when fully resuscitated, the great vessels of the native heart were ligated so that the donor heart assumed the full load. One such animal recovered from anesthetic, stood up, and drank, but died 15 hours later, an event attributed to superior vena caval thrombosis. Other workers were pursuing the same goal but were less successful.

By the early 1950s, it was well established that cardiac transplantation was technically feasible, and studies were undertaken to clarify the physiology of cardiac transplantation. However, the move to orthotopic transplantation had not been achieved, and this was largely due to the difficulties associated with the transfer phase, when the recipient's own heart had been removed, and the problems associated with protection of the donor heart during transfer. These problems were addressed in a report published in 1953 in which the operative technique was simplified by transplanting a heart–lung block, thus reducing the number of anastomotic connections, and the problems of recipient preservation and myocardial protection were solved as both animals were "placed in an ordinary beverage cooler for the production of hypothermia." Using these techniques and arresting the recipient circulation for up to 30 minutes, the authors reported successful transplantation in three dogs, with survival of up to 6 hours.

The recognition of the value of hypothermia as a protective medium was important, but a further step was made toward the possibility of clinical transplantation with the development of the heart–lung machine, pioneered by Gibbon and attributed largely to the technical expertise of the famous pilot Charles Lindbergh. This allowed the circulating blood to bypass completely the patient's own heart and lungs, allowing an extended operative period.

The result of these innovations was that in 1958, the first orthotopic heart transplants were performed, and further steps were taken toward clinical transplantation with the development of a simplified operative technique (Lower and Shumway), which removed the necessity of individual venous anastomoses. The recipient left atrium was circumscribed, leaving a cuff of tissue to sew to the donor left atrium, a relatively simple anastomosis compared with the complex multiple anastomoses of four pulmonary veins. The cavae were reconnected with synthetic tubes, and the arteries were simply sutured end to end. Recipient circulation was maintained with the cardiopulmonary bypass machine, but hypothermia was not required for either donor or recipient. Donor organs were ischemic for between 25 and 32 minutes, and the longest support of circulation by the allografts was 20 minutes.

Further experiments in the late 1950s established that orthotopic transplantation was technically possible, and advances in the surgical techniques used were described. An important paper published in 1960 integrated the developments of the previous decade into a single method for orthotopic transplantation, and five of eight consecutive canine transplant recipients survived for between 6 and 21 days, eating and exercising normally in the postoperative phase. This was the first description of a truly successful procedure in which the circulation was maintained by the transplanted organ.

However, technical ability to perform the transplant operation is clearly not all that is required. In Lower's series, none of the dogs received immunosuppression, and they all died as a result of rapid myocardial failure due to the massive infiltration with round cells and interstitial hemorrhage. Lower and Shumway concluded that "if the immunologic mechanisms of the host were prevented from destroying the graft, in all likelihood it would continue to function adequately for the normal lifespan of the animal."

A further significant step was taken in 1965 when Lower reported the use of the surface electrocardiograph as a marker of rejection episodes. A voltage drop was seen during rejection episodes, which was reversible with the administration of methylprednisolone and azathioprine. With this test as a guide to the intermittent administration of immunosuppressive therapy, survival of 250 days was achieved in an adult dog.

Thus there had been a step-wise progression over the years providing the solution to many of the most difficult problems faced in transplanting the heart, and in 1964, Shumway wrote that "only the immunological barrier lies between this day and a radical new era in the treatment of cardiac diseases." Others clearly felt that the time was already right to undertake cardiac transplantation in man, and a planned approach was made toward this goal at the University Hospital in Jackson, Mississippi, in 1964. Legal and logistic reasons meant that the first man to receive a heart transplant was to receive the heart of a large chimpanzee, and not that of another man. The suture technique of Lower and Shumway was used, and although the operation was technically successful, the heart was unable to maintain the circulatory load, and about 1 hour after cardiopulmonary bypass, attempts at further support were abandoned.

In 1967, the first human-to-human orthotopic heart transplant was performed by Christian Barnard in South Africa. The patient was a 54-year-old man suffering from ischemic cardiomyopathy who received the heart of a 16-year-old female donor. He recovered from the operation but on the 18th day succumbed to pseudomonas pneumonia. On the day that he died, Barnard performed a second transplant, and this recipient survived 594 days.

Following the initial efforts of Barnard in Cape Town and Kantrowitz in New York, 102 cardiac transplants had been performed in 17 countries by the end of 1968. The early results were discouraging, and by 1970, there were only a few centers persevering. Gradually the problems were dealt with, and by 1978 the 1-year survival rate had risen from 22% to 68%, with a return to normal function in 90% of these patients. This was a time of real growth for clinical heart transplantation, with many reports of the early results, infectious complications, and the hemodynamics of the transplanted heart. The indications and contraindications became clearly defined, and donor management was described.

A further great advance was made by Philip Caves, who devised the bioptome for obtaining repeated transvenous endomyocardial biopsies to detect cardiac allograft rejection, and by Margaret Billingham, who described a histological system for grading the rejection reaction seen in these specimens. Further improvements were to be seen with the introduction of rabbit antithymocyte globulin for the prevention and treatment of acute rejection. As the concept of brainstem death became accepted and methods of long-distance procurement were developed, together with donor organ-sharing networks, donor organs became more readily available, ensuring the continued practice of clinical transplantation.

Combined heart and lung transplantation

Demikhov developed a method of heart–lung transplantation in dogs in the 1940s, but it was not revisited until 1953, when Marcus and colleagues at the Chicago Medical School described a technique for heterotopic heart–lung grafting to the abdominal aorta and inferior vena cava in dogs. Disappointingly, however, failure to resume normal spontaneous respiration was noted by a number of groups. Later primates were found to develop a normal respiratory pattern

following complete denervation with cardiopulmonary replacement. A Stanford series showed survival for well over 5 years after heart–lung allograft transplants in primates, allowing Reitz and colleagues to perform the first successful human heart–lung transplant in a 45-year-old woman with end-stage primary pulmonary hypertension in 1981. They utilized a technique that preserved the donor sinoatrial node and eliminated the potential for caval anastomotic stenosis. Subsequently, "domino" transplant was developed, in which the healthy heart of a heart–lung recipient is itself donated for grafting in a cardiac transplant recipient.

Lung transplantation

Experimental lung transplantation developed in parallel with heart–lung transplantation. Metras described important technical concepts, including preservation of the left atrial cuff for the pulmonary venous anastomoses and reimplantation of an aortic patch containing the origin of the bronchial arteries to prevent bronchial dehiscence in 1949. The technique was technically difficult and did not gather widespread acceptance. Transection of the transplant bronchus close to the lung parenchyma was advocated in the 1960s by Blumenstock to prevent ischemic bronchial necrosis. Further surgical modifications to prevent bronchial anastomotic complications included telescoping of the bronchial anastomosis, described by Veith in 1969, and coverage of the anastomosis with an omental flap, described by the Toronto group in 1982. The first human lung transplant was performed in 1963 by Hardy and colleagues at the University of Mississippi; however, the patient only survived for 18 days. It was only in 1986 that the first series of successful single lung transplants with long-term survival were reported from Toronto (with the first patient undergoing transplantation in 1983). En-bloc double lung transplantation was performed by Patterson in 1988 but was later superseded by sequential bilateral lung transplantation, described by Pasque and colleagues in 1990. Subsequently, Yacoub introduced live lung lobar transplantation in 1995.

Indications and refinements

There has been a steady growth in the number of transplants performed, and as transplantation has become more successful in terms of survival, quality of life, and cost benefit, the demand for donor organs has increased so that it is greater than supply. For example, there were 454 thoracic organ transplants performed in the United Kingdom in the year ending December 1992, but at the end of the same year, the number of patients on the waiting lists for cardiac and pulmonary transplantation had grown to 763. Thus even if no more patients were accepted onto the lists, it would take nearly 2 years to clear the back-log of potential recipients. The flaw in this argument is that of these potential recipients, approximately 25–30% will die on the waiting list before suitable organs become available. It is worth noting that the patients who are accepted for transplantation are the tip of the iceberg; many are not referred, and for every patient who is accepted, there are two or three who are rejected, but who might have benefited from transplantation if there were a limitless donor pool.

The annual need for kidneys in the United Kingdom is estimated at between 2500 and 4000, whereas a recent audit of intensive care units in England suggested an absolute maximum number of 1700 potential donors. Even if all these patients were consented for donation and were medically suitable, there would still be a deficit in supply compared with the demand. The demand can be expected to continue to rise, whereas the number of potential donors may be expected to fall as factors such as seat-belt legislation and better trauma care reduce the pool of patients declared brainstem dead.

The indications for transplantation are widening, and although kidney, liver, heart, and even lung transplantation is now seen as routine, the necessary skills are being developed to transplant other organs, such as the small intestine, pancreas, face, hand, and uterus. Clearly this stretches the donor pool beyond its limit.

Other solutions to the donor shortage must be sought if transplantation is to be extended to treat all those in need. Recent trends have seen increased use of living related donors for kidney transplantation, and although renal transplant surgeons have used this resource for a long time, the potential to use livers (first performed in 1989) and lungs from live related donors has only recently been explored. The potential hazards for the donor of such procedures have stimulated fierce ethical debate. Living related donation will never solve the problem entirely, and the fact that such drastic measures can be considered and indeed put into practice underlines the severity of the donor organ shortage.

Another recent development has been the use of organs procured from individuals who die without ever meeting brainstem death criteria. In these patients, once cardiac activity has ceased, kidneys, liver, and even lungs may be removed and used for transplantation as a result of advanced preservation techniques. However, despite the first successful heart transplant being performed using a donor of this nature, there has been no widespread adoption of the non–heart-beating donor for cardiac transplantation.

Organ transplantation may be supplemented or even replaced in due course using totally artificial organs. The only implantable device that finds clinical use at present is the artificial heart. The range of devices available and their apparent complexity underline the difficulties encountered in replacing a relatively simple biological organ with mechanical substitutes. Fundamental problems such as power supply, thrombosis, infection and biocompatibility of mechanical surface-blood interfaces remain, but these obstacles may be overcome in due course to allow long-term function. However, the replacement of those organs with more complex metabolic functions is more difficult, and complete replacements for the kidneys, lungs, and liver are still a long way distant.

The field of organ transplantation has grown massively over the last hundred years. It has been made possible by developments in individual disciplines, supported by growth in our knowledge and understanding of individual organ system physiology and pathology. It remains a challenging and rewarding activity. However, successful as it is, transplantation is not without problems, and it would not be possible at all it were not for the death, often in tragic circumstances, of patients who are suitable for organ donation. Frequently the donors are young people who have met an unexpected accident, or suffered a catastrophic medical event such as subarachnoid hemorrhage, and their death is always an emotionally charged event. Our reliance on the goodwill of the donor's relatives to make available their organs in order that others may live is somewhat perverse, yet it is central to the success of transplantation.

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Section 1 General

Chapter 2

Immunological principles of acute rejection

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Key points

- The immune response to a transplant is a consequence of a complex interplay between the innate and adaptive immune systems.
- The adaptive immune system mounts a highly destructive, sustained, and specific attack on the transplant through recognition of foreign antigens, activation of T cells, expansion of donor-reactive lymphocytes, and infiltration of allografts with effector lymphocytes.
- Immunosuppressive drugs are required to prevent the immune system from destroying the transplant. The majority of immunosuppressants act to inhibit T-cell responses.
- Current immunosuppressive regimens have improved the short-term but not the long-term survival of organ transplants. The broad immunosuppressive activity of these drugs is associated with serious complications, such as an increased risk of malignancies and opportunistic infections.
- An ideal solution to both rejection and the complications of immunosuppression is the induction of tolerance. Research on achieving tolerance clinically is most promising in the fields of mixed chimerism and regulatory T-cell therapy.

The immune system has evolved to clear the host of invading microorganisms and its own cells that have become altered in some way, such as infected cells or mutated tumorigenic cells. The immune system recognizes such cells as "foreign" and the molecules they express as antigens. When organs are transplanted between genetically disparate (allogeneic) individuals, the immune system recognizes and reacts with the foreign antigens of the other individual (alloantigens) on the transplant (allograft) to cause rejection. This rejection response is the result of interplay between the host innate and adaptive immune systems. The innate response is mediated by cells and molecules that include macrophages, dendritic cells (DCs), granulocytes (neutrophils, basophils, and eosinophils), natural killer (NK) cells, and the complement cascade, as well as proinflammatory cytokines and chemokines (chemoattractant cytokines). It represents a preformed defense that is immediately available until a specific response can be mounted by the adaptive immune system. The innate response is less specific than the adaptive response and will be induced even if a transplant has been performed between genetically identical individuals (isograft), simply as a result of implanting or transplanting the cells or organ. Adaptive immunity is mediated by lymphocytes (T and B cells) and displays slower kinetics than the innate response. However, the adaptive response is specific to foreign antigens (alloresponse) and is therefore not activated by isografts. Although the innate immune response is important for the initiation of the alloresponse and can initiate tissue damage, it cannot alone cause rejection (in other words, the complete destruction of the tissue). On the other hand, the adaptive immune response is more damaging and is essential to rejection. The importance of the adaptive response is reflected in the observation that animals experimentally depleted of T cells cannot reject allografts.

This chapter outlines the events involved in the adaptive and innate immune responses to a transplant and the subsequent mechanisms of rejection, concluding with current clinical and experimental strategies to protect transplants from immune-mediated damage.

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Initiation of rejection

The immune system is frequently exposed to harmless (and sometimes beneficial) foreign antigens that do not require an aggressive effector response, such as gut flora. The context in which such foreign antigens are encountered is important in dictating the magnitude of the immune response. For instance, the activation of leukocytes in an inflammatory environment augments the immune response. In transplantation, these inflammatory signals can be provided by the surgical trauma, the oxidative stress of ischemia/reperfusion injury (IRI), and brain death. Indeed, the innate immune response is mediated by cells that express invariant pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), that recognize altered endogenous molecules on the allograft produced as a result of tissue injury by reactive oxygen species (ROS), heat shock proteins (HSP), or high-mobility group box 1 protein (HMGB-1) or as a direct consequence of donor brain death. Activation of innate immune cells by TLR ligation results in the production of "danger" signals such as chemokines and preformed P-selectin (CD62P), which help recruit and direct host leukocytes into the transplant site. Macrophages release cytokines such as tumor necrosis factor (TNF) α, interleukin (IL)-1, and IL-6, which contribute to the inflammatory environment and assist in the activation of other leukocytes. On recognition of inflammatory signals, antigen-presenting cells (APCs) such as DCs in the allograft migrate to the draining lymphoid tissues, where they present antigen to host T cells, leading to an adaptive immune response.

The recognition of foreign antigens by naive host (recipient) T cells (*allorecognition*, otherwise known as *signal 1*) is a principal step in the rejection process. Allorecognition in the presence of costimulation (otherwise known as *signal 2*) results in the activation and expansion of T cells that recognize the mismatched donor alloantigens (alloreactive T cells). Alloreactive T cells orchestrate the development of T cells with effector activity that can either have direct destructive activity against the transplant or promote and amplify B-cell function and other elements of the innate and adaptive immune response that can damage the transplant.

Allorecognition is mediated by the T-cell receptor (TCR), which is associated with the cluster of differentiation (CD) 3 molecule (TCR-CD3 complex). TCRs on host T cells bind to antigens encoded by genes of the major histocompatibility complex (MHC) on donor cells and, to a lesser extent, minor histocompatibility (miH) antigens. In humans, the MHC complex is termed the *human leukocyte antigen (HLA) system*. miH antigens are peptides derived from other molecules that are mismatched between the donor and recipient and are presented by host MHC molecules to host T cells. miH antigens alone cannot cause rapid rejection. However, when multiple miH are mismatched, rejection can be as rapid as when MHC antigens are mismatched. miH mismatches alone may be present in transplants between siblings with identical MHC molecules, leading to slow rejection of these transplants.

There are two pathways by which foreign antigens are recognized by T cells. The more common or natural one is called the *indirect pathway*. Antigens, such as viral antigens, are first processed by host APCs and then presented to host T cells by self-MHC molecules on the APCs. In the transplant setting, the indirect pathway occurs when APCs process and present donor HLA antigens to host T cells within self-MHC molecules. The TCR-CD3 complex on host T cells recognizes unique features of the small processed donor HLA peptides (epitopes) in the context of self-MHC. The second pathway of allorecognition, the direct pathway, is the dominant pathway in transplantation and occurs when T cells react directly with intact donor HLA antigens. By way of comparison, T cells that react to peptides derived from a nominal antigen (indirect pathway) are estimated to be less than 0.1% of the total T-cell repertoire, whereas a much higher frequency (about 10%) of T cells react to an MHC mismatched transplant (direct pathway).

Following organ transplantation, donor-derived "passenger" APCs residing in the donor organ and expressing large amounts of donor HLA antigens migrate out of the transplant into the draining lymphoid tissue, where they interact with host T cells via the direct pathway. With time after transplantation, passenger APCs diminish in number, and the direct pathway becomes less important. In contrast, the indirect pathway of allorecognition is maintained and remains active for as long as the transplant is present. The direct pathway is therefore theoretically more active during acute allograft rejection, whereas the indirect pathway becomes more important later in chronic allograft rejection.

A newly recognized third pathway, called the semidirect pathway, may also be involved in allorecognition. It occurs when intact donor HLA antigens are