

Graft Rejection: Immunological Suppression

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Transplantation of solid organs is the treatment of choice for most patients with end-stage organ diseases. In the absence of pharmacological immunosuppression, recognition of foreign (allogeneic) histocompatibility proteins expressed on donor cells by the recipient's immune system results in rejection of the transplanted tissue(s). One-year renal transplant survival is now routinely over 90% in most centres, largely the result of improvements in immunosuppressive drugs. In this article, we review commonly used immunosuppressive medications and discuss their pharmacological modes of action. Given that long-term graft outcomes remain poor despite improvements in early transplant survival, we discuss, in addition, novel experimental strategies for the induction of tolerance to transplanted tissues that have translational relevance to human organ recipients.

Introduction

Transplantation is the gold-standard treatment for most patients with end-stage diseases of solid organs and the only therapy available where long-term organ support is not available (such as end-stage diseases of the heart and lungs). Tissues transplanted between genetically identical (syngeneic) individuals, such as identical twins, are not recognised as being foreign and are, therefore, accepted (tolerated) without the need for manipulation of the

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Article Contents

- Introduction
- Role of Tissue Typing
- Immunosuppressive Agents
- Reducing Immunogenicity of Grafts
- Induction of Transplantation Tolerance

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immune system. Transplantation of cells, tissues or an organ between genetically nonidentical individuals (allogeneic) in the same species or between different species (xenogeneic) leads to activation of the recipient's immune system and an immunological reaction against the transplant. In this setting, the transplanted tissue(s) (referred to as the 'graft' – Table 1) is destroyed (rejected) if no further intervention is taken. **See also:** [Transplantation](#)

Studies on the behaviour of tumour grafts by Little and Tytzer, among others, led Gorer to propose the concept of graft rejection as long ago as 1938. Recognition that the immune system was responsible came later when Gibson and Medawar clearly identified specificity and memory as hallmark features of the rejection response. Work over the past 50 years has elucidated many of the cells and molecules that are involved, but there is still much to learn. **See also:** [Medawar, Peter Brian](#)

Graft rejection is a complex process. Many factors, including the nature of the tissue transplanted, the genetic disparity – in other words, the histoincompatibility or mismatching between the donor and recipient – the site of transplantation and the immune status of the recipient, all contribute to determine the character of the rejection response. The terms hyperacute, acute and chronic rejection are often used to describe different aspects of rejection responses. **See also:** [Graft Rejection: Mechanisms](#)

Hyperacute rejection occurs when the recipient's immune system has been sensitised to the donor before transplantation. Sensitisation is often accompanied by the presence of antibodies and memory T cells reactive with donor molecules. If the recipient has been sensitised to donor antigens (such as by pregnancy or blood transfusion or a previous transplant), the graft is rejected very rapidly, often within minutes after transplantation. Hyperacute rejection of an allograft occurs only very rarely in clinical transplantation today as transplant recipients are always screened before transplantation to ensure that they have not been sensitised against the graft. Hyperacute rejection is one of the major barriers that need to be overcome before xenotransplantation will be successful, as the vast majority of humans have preformed natural antibodies reactive with

Table 1 Different types of tissue transplantation

Terminology	Definition
Autograft	Tissue transplanted from one part of the body to another (e.g. skin grafts in burns patients and vascular grafts)
Isograft	Tissue transplanted between genetically identical members of the same species (e.g. grafts between identical twins and grafts between members of the same inbred strain of mouse or rat)
Allograft	Tissue transplanted between nonidentical members of the same species (e.g. grafts between genetically disparate humans and grafts between different inbred strains)
Xenograft	Tissue transplanted between individuals of different species (e.g. pig to human and rat to mouse)

pig tissue, in particular a carbohydrate structure that is present in pig but not human cells. **See also:** [Antibodies](#); [Immunological Memory](#); [Natural Antibodies](#); [Xenotransplantation](#)

Acute rejection is the term used to describe the immune response that occurs during the early time period, usually within the first 3–6 months (for kidneys; acute rejection in liver transplantation usually occurs earlier), after transplantation of a genetically mismatched allograft.

Chronic rejection describes the progressive functional deterioration of an allograft occurring months or years after transplantation. This process can often be difficult to distinguish histologically from the scarring processes that characterise transplanted organs exposed to long-term treatment with calcineurin inhibitors (CNIs) (described in the following text) and involves both immunological and nonimmunological factors.

Role of Tissue Typing

Transplants are accepted spontaneously only when the donor and recipient are genetically identical (i.e. identical twins) or if the recipient has significant impairment of immune function (this is rare in a candidate considered appropriate for transplantation). Any degree of genetic disparity or histoincompatibility between the donor and recipient will trigger rejection because the immune system can recognise and respond to the incompatible molecules. **See also:** [Graft Rejection: Mechanisms](#)

Histocompatibility genes and the molecules or antigens they encode are classified as major or minor depending on where the genes are located in the genome. If the gene for a particular histocompatibility antigen maps to the major histocompatibility complex (MHC), the molecule is

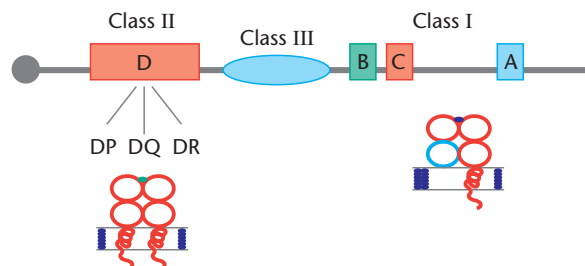


Figure 1 Outline map of genes coding for human leucocyte antigen (HLA) molecules on the short arm of chromosome 6.

referred to as a major histocompatibility antigen or MHC antigen for short. If the gene is encoded outside the MHC, the antigen is referred to as a minor histocompatibility antigen (mHAg). All histocompatibility genes are polymorphic. In other words, many variant forms or alleles of each gene, and hence of the molecule the gene encodes, are present in the population as a whole. **See also:** [Major Histocompatibility Complex \(MHC\)](#)

In humans, the MHC is called the human leucocyte antigen (HLA) complex. As part of the human genome project, the HLA complex has been sequenced (The MHC Sequencing Consortium, 1999). Of the many genes present in the complex, there are two families of genes that code for cell surface molecules known as the HLA class I and II molecules (**Figure 1**). Some of the class I and II molecules have been well characterised and are called HLA-A, HLA-B and HLA-C and HLA-DR, HLA-DQ and HLA-DP, respectively. There are currently approximately 4450 known HLA alleles (please refer to <http://www.ebi.ac.uk/imgt/hla/stats.html> for up-to-date figures). Around 750 HLA-A, 1200 HLA-B and 450 HLA-C functional class I alleles have been described to date. Structurally, HLA class I molecules are composed of an α chain and a non-covalently associated β 2-microglobulin chain. Although there is considerable polymorphism in the α chain, the β 2-microglobulin subunit is not polymorphic.

For HLA class II molecules, where the genes for both the α and β chains of each molecule (known as A and B genes, respectively) are encoded by the MHC (**Figure 1**), around 600 HLA-DRB1, 3 HLA-DRA, 75 HLA-DQB1, 25 HLA-DQA1, 120 HLA-DPB1 and 15 HLA-DPA1 alleles have been identified. HLA class II has considerable polymorphisms in both α and β chains (except in the HLA-DR α chain, where there are currently only three allelic forms described so far) in contrast to HLA class I.

The techniques of tissue typing are used to identify the combination of HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and HLA-DP alleles that are present in any one individual. The combination of alleles is often described as the individual's tissue or HLA type. **See also:** [Major Histocompatibility Complex: Human](#)

An important change to the nomenclature of HLA typing has come into effect as of April 2010 to accommodate the growing number of HLA antigens. Under the

new system, HLA nomenclature will be changed such that, for example, HLA-A*0201 becomes HLA-A*02:01, allowing for HLA-A*02:101 etc. In this way, greater numbers of HLA types can be accommodated in the same nomenclature system. For a more complete description, please see <http://hla.alleles.org/announcement.html> or refer to Marsh *et al.* (2010).

Matching the organ donor and recipient for HLA antigens has a marked benefit on graft survival. The degree of HLA matching required is dependent on the tissue or organ transplanted. For bone marrow transplants, it is critically important to match the donor and recipient for all typed HLA molecules. For this reason, large registries of millions of people who have been tissue typed and are willing to act as bone marrow donors have been established. In this way, when a patient needs a bone marrow transplant, a donor who is as closely matched as possible can be found very quickly. **See also:** [Tissue Typing for Transplantation Antigens](#)

For recipients of solid organ grafts, such as kidney, heart and liver, the effects of HLA matching – or more correctly mismatching – on graft survival are organ dependent. Until recently, all donor–recipient pairs had to be matched for the ABO blood group; in some centres now, planned ABO incompatible transplantation (especially of kidneys) is being carried out following plasma exchange of recipients before transplantation to remove antibodies against donor blood groups.

Most centres focus on the tissue type of the donor and the recipient at three loci: HLA-A, HLA-B and HLA-DR for matching purposes. For recipients of a transplant from a living donor, excellent graft survival is seen when the recipient and donor are matched for HLA. When compared to cadaveric donors, a greater degree of mismatch for living donation is acceptable as, despite a higher frequency of acute rejection episodes, graft outcomes are still better (reflecting nonimmunological determinants such as the length of ischaemic injury, age of the graft etc.).

Although excellent graft survival is also achieved with organs from cadaver donors when they are fully HLA matched with the recipient, this degree of matching would be possible for the majority of patients only if organs were shared between centres worldwide. As this would be impractical, it is fortunate that many studies have shown that excellent graft survival can be achieved with modern immunosuppressive drugs when the donor and recipient are matched for some but not all HLA antigens. When graft survival data are analysed, a hierarchy in the ‘strength’ of the different HLA loci to trigger rejection can be identified. HLA-DR antigens have been shown to be the ‘strongest’ triggers of rejection, followed by HLA-B and HLA-DQ. Analysis of survival data for kidney allografts collected by different transplant centres around the world has shown that if the donor and recipient are mismatched for HLA-DRB1, this has a negative effect on graft survival (**Figure 2**; Morris *et al.*, 1999). In other words, graft survival is less good in long term in patients who receive a kidney from a donor mismatched for HLA-DRB1 than in patients who receive a kidney from a donor who is matched for

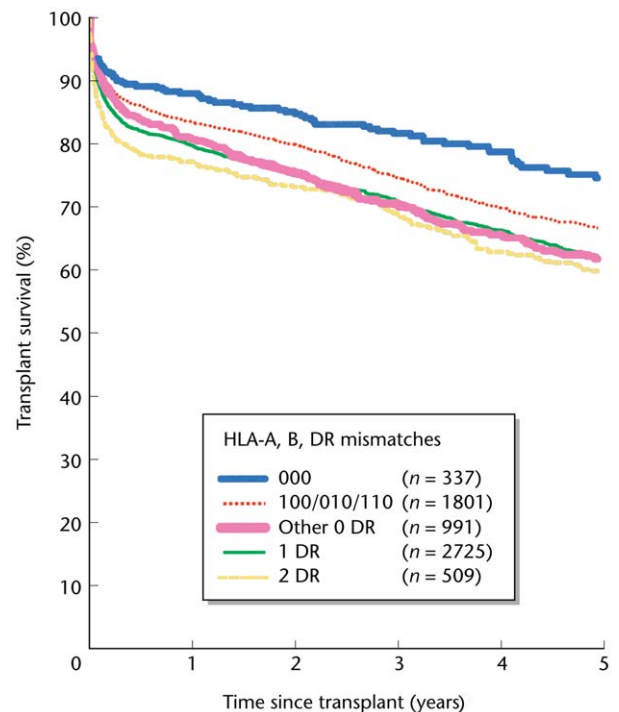


Figure 2 Transplant survival rate in recipients mismatched for donor human leucocyte antigen (HLA) A, HLA-B and HLA-DR. Reproduced from Morris *et al.* (1999), with permission.

HLA-DRB1. Analyses of graft survival data can be found on several transplantation websites (described in the following text). **See also:** [Histocompatibility Antigens](#); [Immunosuppressive Drugs](#)

Many mHAg systems must exist in humans, and their influence on graft rejection may be significant (Simpson *et al.*, 1998). For example, a small number of kidney grafts transplanted between HLA-identical siblings undergo rejection episodes, which occasionally lead to graft loss. Differences in mHAg between the donor and recipient are thought to trigger rejection in this situation. In general, mHAg can be classified based on the cells of origin (Afzali *et al.*, 2007):

- (i) Encoded by sex chromosomes – a set of proteins encoded by genes on the male Y-chromosome (the H-Y antigens).
- (ii) Encoded by autosomes – non-Y-chromosome encoded mHAg including the HA antigens (named after the initials of the first patient who developed graft-versus-host disease after bone marrow transplantation as a result of mHAg mismatches) among others.
- (iii) Encoded by mitochondrial DNA (mitochondrial deoxyribonucleic acid, mtDNA) – mtDNA is, by definition, maternally inherited and, therefore, such peptides could act as histocompatibility antigens.

In bone marrow transplantation, mismatching for minor antigens can lead to graft-versus-host disease. However, in

general, minor antigens remain poorly characterised, in particular the impact of mHAg mismatching for solid organ graft survival. For an update on mHAgs in solid organ transplantation, refer to the article by Dierselhuber and Goulmy (2009).

Immunosuppressive Agents

Immunosuppressive agents are used to control the immune response after transplantation of an HLA-mismatched graft. If no immunosuppression is used, the graft will be rejected. After transplantation, patients need to take immunosuppressive drugs continuously to ensure that the immune system is adequately suppressed, allowing the graft to survive and function for as long as possible. From the early 1960s, azathioprine (Aza), a relatively nonspecific inhibitor of cell proliferation, and steroids, which are anti-inflammatory, provided the basis for immunosuppressive therapy in clinical renal transplantation. Subsequently, a number of new immunosuppressive drugs have been developed such that today transplant patients are treated with a cocktail of immunosuppressive agents to ensure that the immune response to the graft is very tightly controlled throughout the posttransplant course. The development of drugs for use in clinical transplantation is outlined in **Table 2**. **See also:** [Immunosuppression: Use in Transplantation](#); [Immunosuppressive Drugs](#)

Different immunosuppressive drugs target the immune response at various points as it develops after transplantation (**Figure 3**). As a result, some of the drugs can be used

effectively in combinations to try to target the response at multiple points to ensure that the immunosuppression achieved is as effective as possible and to minimise the dose of each drug that is needed (thereby reducing incidence and severity of adverse reactions).

The CNIs, cyclosporin A (CsA) and tacrolimus, used in combination with other agents, are the mainstay of modern transplant immunosuppression. CsA was first shown to have potent immunosuppressive properties by Borel and colleagues in 1976 and, as a result of promising data from the early clinical trials, it was developed for clinical use. Although CsA is a potent immunosuppressive drug, it is not without side effects, the most serious of which is nephrotoxicity. As a consequence, all newer immunosuppressive protocols that use cyclosporin in combination with other drugs are designed with the aim of using lower doses of cyclosporin to reduce the incidence of nephrotoxicity. CsA works by binding intracellularly to the immunophilin, cyclophilin, a molecule that normally plays a role in protein folding. The CsA–cyclophilin complex then binds to the calcineurin–calmodulin complex and inhibits the phosphorylation of a transcription factor, NF-AT (nuclear factor of activated T cells). NF-AT is required for transcription of genes whose products, including interleukin 2 (IL-2), play a role in ‘early’ T-cell activation. CsA therefore acts to block T-cell activation at a very early point in the triggering process (**Figure 3**). **See also:** [T-lymphocyte Activation](#); [T Lymphocytes: Helpers](#)

Tacrolimus, still often called FK506, the name the drug was given when it was first investigated, acts at a similar point in the cell cycle to CsA (**Figure 3**). Consequently, it is

Table 2 Development of immunosuppressive agents that are in clinical use

	Mechanism of action
<i>1955–1965</i>	
Steroids	Anti-inflammatory
Azathioprine	Antiproliferative
<i>1965–1975</i>	
Polyclonal antithymocyte globulin (ATG) or antilymphocyte globulin (ALG)	Leucocyte depletion
<i>1975–1985</i>	
Cyclosporin A	Inhibits IL-2 gene transcription
<i>1985–1995</i>	
Anti-CD3 monoclonal antibody	T-cell activation, opsonisation and depletion
Tacrolimus	Inhibits IL-2 gene transcription
Mycophenolate mofetil	Inhibits IMPDH
<i>1995 to present</i>	
Anti-CD25 monoclonal antibodies (IL-2R α chain)	Inhibits IL-2 function
Sirolimus	Inhibits cytokine-mediated signal transduction
Anti-CD20 monoclonal antibody (chimaeric)	Targets B cells

Notes: IL, interleukin and IMPDH, inosine monophosphate dehydrogenase.

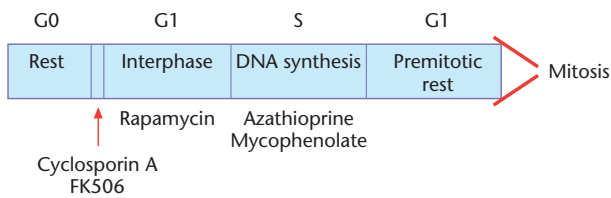


Figure 3 Stages of the cell cycle affected by immunosuppressive drugs.

also an inhibitor of T-cell proliferation but it is approximately 100 times more potent than CsA. Tacrolimus binds to the immunophilin FK-binding protein 12 (FKBP12) within the cytoplasm of the cell. This drug-immunophilin complex can also bind to the calcineurin–calmodulin complex, and results in the inhibition of transcription factor activity as described in the preceding text. As might be expected, tacrolimus has been reported to have a similar side effect profile to CsA, including nephrotoxicity and neurotoxicity, but also has a relatively high incidence of causing posttransplant diabetes mellitus. Tacrolimus is used routinely in many centres in preference to CsA and in others as an alternative to CsA where immunological risk is high such as an HLA-sensitised patient. In liver transplant recipients, tacrolimus is the CNI of choice. Tacrolimus has also been used with considerable success to rescue patients who are experiencing rejection that is resistant to the action of steroids and/or antilymphocyte agents (antithymocyte globulin (ATG) or OKT3).

Sirolimus (also known as rapamycin after its discovery on the Polynesian island of Rapa Nui) is another immunosuppressive agent that acts as a potent inhibitor of T-cell proliferation. It was originally developed as an antifungal agent belonging to the macrolide family. Although rapamycin binds to the same immunophilin as tacrolimus, FKBP12, sirolimus does not block the transcription of early activation genes such as IL-2 but, rather, disrupts the IL-2 receptor (IL-2R) signal transduction pathway – the rejection response downstream of IL-2 production. Its effects are, therefore, at a later time point in the cell cycle (**Figure 3**), and importantly, result in inhibition of both T- and B-cell activation and maturation. The difference between sirolimus and tacrolimus arises owing to the fact that binding of sirolimus to FKBP12 leads to inhibition of the mammalian target of rapamycin (mTOR) instead of calcineurin. This difference also explains a different side effect profile of sirolimus compared to the CNIs (no nephrotoxicity, but impaired wound healing, thrombocytopenia and metabolic side effects). Sirolimus may also have a role to play in the treatment of conditions other than transplantation, including tuberous sclerosis complex and as an adjunct in cancer chemotherapy. Some centres also use sirolimus coated stents in the treatment of coronary artery stenosis (taking advantage of its antiproliferative effects).

Mycophenolate mofetil (MMF) is a potent immunosuppressive agent that is converted to mycophenolic acid, its active metabolite, in the liver and that inhibits the

enzyme inosine monophosphate dehydrogenase (IMP DH), thereby preventing DNA synthesis. Lymphocytes rely on *de novo* purine synthesis for replication whereas other cells can utilise the ‘scavenger pathway’ and recover purines required for cell division. MMF can therefore be used to inhibit polyclonal proliferative responses of both T and B cells, and prospective randomised clinical trials of MMF have shown that it can be used to prevent acute rejection of solid organ grafts. Owing to its mode of action, MMF also causes bone marrow suppression and gastrointestinal adverse effects (mainly nausea and diarrhoea), so regular blood tests are required when on this drug to ensure that excessive bone marrow suppression does not occur. In contrast to MMF, Aza, another antiproliferative agent, works in a related fashion to MMF. Aza is converted *in vivo* to 6-mercaptopurine (the version under which it was first introduced as a drug by Sir Roy Calne) and incorporated into DNA, causing feedback inhibition of the purine synthesis pathway. Not surprisingly, Aza inhibits division of rapidly proliferating cells, including T cells and bone marrow, therefore its side effect profile includes marrow suppression and opportunistic infections. In many centres, MMF has overtaken Aza as the antiproliferative of choice owing to a more favourable side effect profile, in particular with respect to long-term development of skin cancers and opportunistic infections.

All of the agents mentioned in the preceding text are small chemical immunosuppressive molecules. In addition to the use of these agents to prevent graft rejection, larger so-called ‘biological’ molecules are used. These include polyclonal and monoclonal antibodies that target lymphocytes (**Table 1**). Polyclonal antithymocyte or antilymphocyte globulin (ATG and ALG) has been used for many years to treat acute rejection when it occurs. ATG contains a collection of different antibodies that recognise molecules present on the surface of human lymphocytes. It is infused into transplant patients during a rejection episode and has the effect of eliminating or depleting lymphocytes. As the lymphocytes are known to be the major cellular mediators of rejection, their elimination should result in immunosuppression. These drugs need to be monitored carefully with serial blood tests as they can cause profound lymphopenia. To try to make this type of rejection treatment more selective, monoclonal antibody preparations have been developed recently; these include antibodies anti-CD3 and anti-CD25. **See also: Monoclonal Antibodies: Therapeutic Uses**

CD3 is expressed by T cells. The monoclonal antibody, OKT3, recognises and binds to cells expressing CD3, targeting them for activation, opsonisation and lysis. OKT3 can be used to treat patients undergoing their first acute rejection episode after renal (Ortho Multi Centre Study Group, 1985), liver or heart transplantation. OKT3 has also been used by some centres for prophylaxis or ‘induction’ therapy with the aim of improving long-term allograft survival by delaying the first episode of acute rejection. The administration of OKT3 is not without side effects. The majority of patients treated with the monoclonal antibody

experience transient flu-like symptoms due to cytokine release as a result of activation of the T cells targeted by the antibody. In addition, this monoclonal antibody was of mouse origin (i.e. a xenogeneic protein). When it was used as a therapeutic agent, most patients made an immune response against the mouse protein that neutralised the biological effect of the antibody.

The technology used to generate monoclonal antibodies has progressed markedly since the introduction of OKT3 into clinical use. It is now possible to engineer antibodies using molecular techniques such that an antibody with the desired binding reactivity can be made to resemble a human antibody as closely as possible – humanised or chimeric monoclonal antibodies (Winter and Milstein, 1991). In this way, when the antibodies are used as therapeutic agents the protein infused is not xenogeneic, thus reducing the possibility of triggering an immune response. CD25 is the α chain of the IL-2R. It is expressed by lymphocytes only once they have been activated. Engineered monoclonal antibodies targeting the CD25 molecule have been developed, and in many centres are used as induction immunosuppression for transplantation. Similarly, chimeric anti-CD20 is used in some centres. Its mechanisms of function, apart from B-cell depletion, are incompletely understood, and diverse activities, including down-regulation of the B-cell receptor (BCR), have been demonstrated. The exact niche for anti-CD20 has yet to be fully explored; however, most centres reserve its use for induction therapy in highly sensitised individuals to transplant antigens (including ABO blood group incompatible transplants), antibody-mediated rejection and for the treatment of rejection resistant to other therapies. The use of anti-CD20 as induction therapy is currently contentious given a report that giving this agent on the day of transplantation actually increases rates of acute rejection (this study was terminated early as a result; Clatworthy *et al.*, 2009). Nevertheless, other reports for the use of anti-CD20 in transplantation have been more favourable, with a Swedish study showing a reduction in acute rejection risk when using this agent before transplantation (Tyden *et al.*, 2009). Anti-CD20 is currently under investigation (the RituxiCan study) as a treatment for chronic allograft nephropathy. **See also:** [Monoclonal Antibodies](#)

In most transplant centres, the immunosuppressive drugs aforementioned are used in combination with each other, allowing targeting of different pathways and minimisation of dose of each agent. The first 3–6 months (depending on the type of transplant) posttransplantation are immunologically the greatest at risk period for acute rejection. Therefore, standard practice is to transplant the patient with relatively high dose of at least two but, more commonly three, agents (e.g. tacrolimus, MMF and steroids) with an induction agent, usually a monoclonal antibody to deplete circulating T cells (e.g. anti-CD25 treatment). Dosages of the three drugs are then monitored over time and gradually weaned. Long-term immunosuppression depends on local practice from centre to centre but could include anything from one to three agents at low dose.

Although the immunosuppressive agents described in the preceding text are very effective in the short term, they all have both immunological and nonimmunological side effects directly or indirectly associated with their use in transplant patients. These side effects can compromise both the function of the transplant and the quality of life of the transplant patient. All the immunosuppressive agents currently in clinical use act on the immune system non-specifically. In other words, instead of just targeting those elements of the recipient's immune system that are activated after transplantation and that play an active role in the immune response against the transplant, the drugs suppress the whole immune system nonselectively. This means that transplant patients are less able to mount effective immune responses against infection and have an increased risk of developing cancer. Immunosuppressive agents that target the immune system more selectively, and ultimately, specifically, resulting in donor-specific unresponsiveness or tolerance, will improve this situation. **See also:** [Immune System](#)

One-year graft survival rates have improved remarkably since the earliest days of clinical renal transplantation such that at present most centres report survival figures of over 90% for kidney grafts at 1 year. Unfortunately, this remarkable short-term improvement in graft survival has not resulted in a corresponding increase in long-term graft survival. Half of kidney transplants still fail within 8 years of transplantation, and the rate of graft loss after the first year has not changed in the past 20 years. This illustrates very clearly that the immunosuppressive agents currently available either do not control the immune system effectively, and are unable to prevent chronic rejection, or have adverse effects directly on the graft (nephrotoxicity for example). Of particular note, the most common cause of graft loss in the long term remains death of the patient with a functioning graft. This is usually the result of an excess of cardiovascular disease, which is associated with adverse effects from transplant immunosuppression. Nevertheless, for the vast majority, long-term outcomes for patients are still better if they have a transplant than if they remain on dialysis.

Reducing Immunogenicity of Grafts

To reduce the requirement for immunosuppressive drugs, attractive options include either reducing immunogenicity of the graft or inducing a state of immunological tolerance to the transplant in such a way that the patient selectively ignores the graft but continues to respond normally to other antigens (such as infectious agents).

Donor-derived passenger leucocytes, immature dendritic cells, are present within solid organ grafts at the time of transplantation. These cells are triggered to migrate out of the graft as a result of the inflammation caused by removing the organ from the donor and transplanting it into the recipient. When the donor passenger cells migrate from the graft to the recipient lymphoid tissue, they change

their functional properties and become potent antigen-presenting cells (APCs; Banchereau and Steinman, 1998). As a result, they can present the donor histocompatibility antigens that are mismatched to the recipient immune system, thereby triggering rejection. One way of potentially reducing the immunogenicity of a graft would be to eliminate the passenger leucocytes before transplantation. These data support the idea that the immune system requires two signals when it recognises antigen to become activated (Lafferty *et al.*, 1983). If only one signal is presented on encounter with donor antigen, the immune system will not be activated, and under the correct circumstances, will actually be inactivated and fail to respond. In support of this hypothesis, when kidney grafts that have been transplanted into immunosuppressed recipients are retransplanted into a second naive recipient, they survive without immunosuppression. After transplantation into the primary host, the donor-derived passenger leucocytes present in the graft would have migrated to the recipient lymphoid tissue. When the grafts depleted of passenger leucocytes were retransplanted, they were less immunogenic and unable to trigger rejection. To confirm that the absence of donor-derived passenger leucocytes was responsible for the prolonged graft survival in the second recipient, donor APCs were infused at the time of retransplantation. In this situation, the kidneys were rejected. **See also:** [Antigen-presenting Cells](#); [Dendritic Cells \(T-lymphocyte Stimulating\)](#); [Lymphocyte Activation Signals](#); [Transduction](#); [Lymphocytes: Antigen-induced Gene Activation](#)

Removal of donor-derived passenger leucocytes from solid organ grafts presents a challenge that is orders of magnitude more difficult than their removal from cellular grafts such as islets of Langerhans. When the passenger cells are eliminated, islet grafts are less immunogenic and survival is prolonged, in some experimental studies indefinitely without nonspecific immunosuppression.

Induction of Transplantation Tolerance

In transplantation, the term tolerance is taken to mean the continued survival and function of a graft in the absence of a deleterious immune response and chronic immunosuppression. The ability to switch off, or even modify, the immune response specifically to the alloantigens expressed by the organ donor without compromising the recipient's ability to respond to other immune challenges after transplantation would represent a major advance in clinical transplantation as we now know it. As aforementioned, increasing the specificity of immunosuppression required to inhibit the immune response against the transplant would result in a significant reduction in the adverse consequences of a lifetime of immunosuppression. Moreover, if xenotransplantation is to become a routine clinical procedure, the induction of tolerance may have to become an

essential part of any treatment protocol, and in this situation the induction of T- and B-cell tolerance may be essential. **See also:** [Immunological Tolerance: Therapeutic Induction](#)

In straightforward terms, the strategies that are being explored for the induction of transplantation tolerance fall into three broad categories: (1) strategies that rely solely on the deletion of donor-reactive lymphocytes, (2) strategies involving induction of either a suppressor or regulatory population of lymphocytes (that can control the immune response against the transplant; Sakaguchi *et al.*, 2008) *in vivo* or expanding these cells *ex vivo* for reinfusion into the recipient of the transplant (Sagoo *et al.*, 2008) and (3) strategies that invoke both mechanisms stimulating apoptosis or programmed cell death of T cells in the early post-transplant phase and the development of regulatory T cells (Tregs) in the longer term.

Mixed allogeneic chimaerism is one approach that can be used to delete donor alloreactive or xenoreactive lymphocytes *in vivo* (Sykes and Sachs, 1988). In this system, the transplant recipient is manipulated using biological agents that target T-cell function either alone or in combination with low-dose irradiation before infusion of a mixture of bone marrow cells from both the recipient and organ donor. This results in the development of long-term, stable, mixed, allogeneic chimaerism in the recipient and deletion of donor-reactive lymphocytes from their immunological repertoire. For this approach to be used successfully in clinical practice, the ability to achieve engraftment of haematopoietic tissues without ablative treatment of the recipient is essential. With an increased understanding and new insights into stem cell biology, cell migration *in vivo* and growth requirements for haematopoietic cell engraftment may become easier to achieve in the future.

New reagents for depleting peripheral leucocytes more effectively are being developed. Data using an anti-CD3 immunotoxin to manipulate the peripheral T-cell repertoire before transplantation have shown that this can lead to the long-term survival of renal allografts in primates, with tolerance to donor alloantigens developing in some recipients (Knechtle *et al.*, 1997). The principles highlighted by these experiments have stimulated a number of other studies (Calne *et al.*, 1998) that may result in the identification of an effective strategy that can be used clinically. **See also:** [Immunotoxicology](#)

Work on novel approaches for developing peripheral tolerance is progressing rapidly as new targets for manipulating immune responses with biological agents are identified. Biological agents that target CD3, CD4 and CD8 molecules have all been shown to induce tolerance to alloantigens in experimental models (Waldmann and Cobbold, 1998). Blockade of costimulation by targeting the CD28–CD80/CD86 and/or the CD40–CD154 pathways is also producing exciting and impressive experimental findings (Harlan and Kirk, 1999). The majority of these approaches lead to the development of immunoregulation specific for donor antigens *in vivo*. The characteristics of the leucocytes responsible for

immunoregulation are being defined, and this information will be invaluable for refining these approaches in the future. These same agents may also facilitate stem cell engraftment of haematopoietic cells which would lead to deletion of donor-reactive cells. More targets will present themselves as our understanding of the pathways for costimulation and immunoregulation *in vivo* increases. The potential of CD152 (cytotoxic T lymphocyte-associated antigen 4, CTLA-4), to downregulate immune responses is intriguing (Bluestone, 1998), and exploration of the molecular mechanisms involved is certain to focus attention on this as a possible way of controlling immune responsiveness to transplant and developing tolerance in the future. Similarly, trials of anti-CD20 in the context of transplantation are planned to determine whether this therapeutic intervention has the capacity to induce donor-specific regulatory mechanisms and help minimise steroid and CNI exposure. **See also:** [Immunoregulation](#); [Transplantation of Haematopoietic Stem Cells](#)

The use of biological agents such as monoclonal antibodies or soluble recombinant ligands at the time of transplantation to facilitate the development of long-term graft survival and ultimately tolerance is also not without difficulty and presents many challenges of its own. One unresolved issue is how to use biological agents effectively in combination with conventional immunosuppressive drugs such as cyclosporin, tacrolimus and MMF. Data from experimental studies suggest that the use of a biological agent and cyclosporin simultaneously at the time of transplantation may inhibit the development of long-term graft survival (Larsen *et al.*, 1996). If this finding is reproducible, the identification of ways in which the biological agents can be combined effectively with immunosuppressive drugs is essential. Work on this topic is already in progress, and before too long new insights should emerge into the way the intracellular pathways affected by the drugs and those required for the induction and maintenance of tolerance intersect.

Newer approaches use pretransplant administration of alloantigen in combination with biological agents with the objective of developing specific unresponsiveness to a defined set of alloantigens before transplantation. In this way, the mechanisms responsible for the development of the unresponsive state should be established before transplantation and the administration of immunosuppressive drug therapy.

Induction of tolerance using cell therapy has also become a possibility in the last few years. The discovery of a population of professional suppressive T cells, known as Tregs, which express CD4 and high levels of the IL-2R α chain, have made this a much more realistic possibility. Tregs arise from the thymus (naturally occurring or nTregs) and account for approximately 2–5% of the peripheral CD4⁺ T cell pool. They can also be induced to develop from conventional T cell under the appropriate milieu (induced or iTregs). This milieu includes divergent signals such as antigen concentration, cytokine environment, availability of costimulation and, by extension, APC

type and maturation state. Physiologically, Tregs are critical for maintenance of tolerance to self-components and prevent the development of autoimmune diseases. In animal models, Tregs can prevent transplant rejection and also ameliorate autoimmune diseases. Two broad possibilities for Treg therapy exist in humans, namely induction of Tregs from naïve precursors *in vivo* using targeted delivery of alloantigen under tolerising conditions (appropriate cytokines, immunomodulatory monoclonal antibodies, costimulatory blockade etc.) or infusion of autologous Tregs expanded from peripheral blood *ex vivo*. Recently, it has been demonstrated that Tregs can be expanded *in vitro* from healthy human blood and retain their suppressive function. This process is more efficient if the Tregs are cultured *ex vivo* with sirolimus. Although more work is required to establish the safety of using expanded Tregs in human patients, administration of Tregs to transplanted patients to induce tolerance to their graft and allow dose reduction in their immunosuppressive drugs is a realistic target over the next few years.

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