Transplantation: drug aspects of immunosuppression

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The first article in our special feature explains the principles of immunosuppression to prevent rejection of a transplanted organ and discusses how the different types of immunosuppressant drugs are used in clinical practice.

**Introduction**

The first successful transplant took place at the Brigham hospital in Boston in 1954. The organ (allograft or graft) was a kidney, and it was transplanted between genetically identical twins. Fifty years of immunological, surgical and pharmaceutical advances have led to solid organ transplantation becoming almost a routine option for some indications. In the year to 1 April 2002, transplantation of a liver, heart or lungs have led to solid organ transplantation of 1,685 patients with end-stage renal disease the prospect of a better quality of life compared with dialysis. Most kidney recipients are able to function within society at what is considered a normal level. Health systems also benefit from kidney transplantation as it is a significantly more cost-effective treatment than any type of dialysis.

**Immune Responses**

By the early 1950s, it was understood that when an identical twin donates an organ the recipient will not invoke an immune response against the “foreign” tissue. In all other cases, recognition of non-self by a recipient’s T- and B-lymphocyte mediated, adaptive immune system leads to a variety of responses. This is called transplant rejection and preventing it has been a major focus of attention. To understand how prevention might be achieved, a basic explanation of the cellular processes involved is required.

If a potential patient has previously been exposed to non-self, human antigens (for example, through a blood transfusion, prior transplant or even pregnancy) this may have resulted in the presence of pre-formed antibodies. If these match an antigen from a donor, then hyperacute rejection, which ensues within minutes or hours, will irreversibly damage the donor organ and lead to loss of the allograft. To prevent this catastrophic event, a pre-transplant cross-match of donor lymphocytes with recipient serum is usually undertaken. Ensuring ABO blood group compatibility is routine for all organ transplantation.

Varying severities of acute rejection can occur at any time post-transplant but are most common in the first few months. Donor molecules that are expressed on the surface of graft cells (for example, human leukocyte antigens [HLA]) are presented, on antigen presenting cells, to resting CD4+, T-help lymphocytes that have a receptor for that antigen. The recognition process activates these T-cells to produce and secrete chemical signals (interleukin-2 [IL-2] is considered the main cytokine) and also to express cell-surface receptors to them (eg, interleukin-2 receptor [IL-2R]). This line of T cells then differentiates and proliferates under the influence of these chemical messengers. Through this process, CD8+, or cytotoxic, T cells are activated to lyse the donor cells. B lymphocytes are activated by other interleukins, leading to antibody formation. This intense, local immune activity prompts the involvement of the innate immune system with its phagocytic cells and enzyme systems, such as those comprising complement pathways. In the acute cellular rejection of kidneys, biopsy usually demonstrates T-cell influx. The higher grade, acute vascular rejection is thought to involve a greater degree of antibody deposition. Untreated acute rejection will result in graft loss.

Chronic rejection can occur in any donor organ as early as just a few months post-transplant, though it is most common after a number of years. The onset and progress of graft deterioration is slow and relentless. Arteriosclerosis is seen on biopsy in most organs. The aetiology is not fully understood and many factors, including cytomegalovirus infection, may contribute to the immune response. In kidney transplants, the term “chronic allograft nephropathy” is often used in recognition that an element of this process might be related to the nephrotoxicity of some immunosuppressants. What is clear is that episodes of acute rejection are risk factors for the subsequent development of chronic rejection.

**Drug Aspects of Immunosuppression**

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**Conclusion**

The principles of immunosuppression and the different types of immunosuppressant drugs used in clinical practice are discussed. This feature explains how the different types of immunosuppressant drugs are used in clinical practice.
two approaches are used to limit the potential for a graft to be lost to rejection. All possible steps are taken to maximise the compatibility of organ and recipient. With kidney transplants, HLA typing is also undertaken in advance to match donors as closely as possible to genetically compatible recipients. This reduces the frequency of rejection episodes and results in better outcomes. HLA tissue typing is not routine upon centre preference. This will also be a predictor of graft survival.11

The main adverse effect of azathioprine is a reversible, dose-dependent suppression of bone marrow. Concurrent use of drugs that inhibit xanthine oxidase (eg, allopurinol), a major elimination pathway for thiopurines, requires the azathioprine dose to be reduced by around 75 per cent if marrow suppression is to be prevented. Recent large studies have shown that around one in five patients experiences early nausea attributed to azathioprine.17 If used with other gastric irritant drugs, such as steroids, the doses are generally separated and given after meals. Other adverse effects are rarely seen.

A thiopurine was one of the first drugs used to prevent graft rejection. It is licensed for combination use in renal, hepatic and cardiac transplantation. It is usually given as a once daily, oral dose of 1 to 2mg/kg/day unless a patient is nil by mouth when intravenous infusion may be used.

The drug is well absorbed and is quickly metabolised to its parent compound, 6-mercaptopurine (6-MP). The thioguanine nucleotides which result from further degradation of 6-MP disrupt the cellular synthesis of RNA and DNA. Preventing mitosis, and thus the proliferation of activated T and B cells, has been a key part of most anti-rejection strategies.

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Renal Registry Data identified a reduced incidence of chronic allograft nephropathy with mycophenolate, suggesting that the acute rejection advantage might benefit graft survival. Enenteric coated mycophenolate sodium is expected to be launched soon.

MPA is a non-competitive, selective, reversible inhibitor of the enzyme inosine monophosphate dehydrogenase. This enzyme is the crucial, rate-limiting step in the synthesis of guanosine nucleotides. Unlike most other cell lines, lymphocytes do not have alternative pathways for producing these DNA building blocks. Mycophenolate prevents T-cell proliferation. It is then metabolised to an inactive glucuronide conjugate that is excreted via the kidneys.

Mycophenolate mofetil is commonly associated with gastrointestinal and haematopoietic adverse effects. In trials it has caused more diarrhoea and vomiting than azathioprine but less nausea. Dyspepsia is commonly reported by patients and may be ameliorated by splitting the total dose, giving it four times a day. Concurrent administration of magnesium or aluminium antacids impairs MPA absorption, which might compromise treatment. The incidence of leucopenia with mycophenolate mofetil is slightly lower than with azathioprine.

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**NEW AGENTS**

Sirolimus is the latest immunosuppressant to be licensed to prevent renal transplant rejection. It is recommended that after an initial three-month combination with ciclosporin and steroids either the sirolimus or ciclosporin is withdrawn. Studies have shown sirolimus to be as effective in terms of graft survival and preventing acute rejection as Neoral ciclosporin, when used in standard regimens. Although it has a key advantage of not being nephrotoxic there is as yet no consensus on the role of this drug in drug regimens. This is in part because sirolimus was used in very different ways in the phase III studies. Increasingly, evidence suggests that, at least in kidney transplants, the drug might best be used to replace ciclosporin or tacrolimus to maximise renal function.

In the US, the Food and Drug Administration has recommended that sirolimus is not used in lung transplant because of the incidence of dehiscence of the bronchial anastomosis seen in investigator-led studies. Further, excess mortality and an increased incidence of hepatic artery stenosis have been identified from de novo use in combination with tacrolimus and ciclosporin in liver recipients.

Trough, whole blood drug monitoring is recommended, with lower levels being sought when combined with ciclosporin (4-8ng/ml) than when used alone (12-20ng/ml). Monitoring should be performed not less than one or two weeks after a dose change as sirolimus has an elimination half-life of around 60 hours.

Everolimus, a similar agent with the same mechanism of action, will shortly be launched in the UK.

In the sense that these drugs impair the ability of lymphocytes to proliferate, they might best be considered alongside the anti-metabolites. However, it is likely that side effect profiles and new clinical data will dictate that they replace ciclosporin or tacrolimus. Once bound to an intracellular protein (FKBP-12), both sirolimus and everolimus inhibit a protein kinase [mammalian target of rapamycin (m-TOR)], thereby blocking a cell's ability to respond to the cytokine signals to progress through the cell cycle and proliferate. In addition, both these drugs inhibit the remodelling of vascular smooth muscle and intimal thickening which is commonly seen in biopsy of grafts with chronic rejection.

The main dose-related adverse effects attributable to sirolimus are hyperlipidaemia, thrombocytopenia, leucopenia and hypokalaemia. Delayed wound healing and a high incidence of lymphocele are strong arguments for delaying introduction until well after the post-operative period.

Arthralgia occurs in up to 20 per cent of patients. Unlike ciclosporin and tacrolimus, sirolimus is not associated with hirsutism, gain hypertrophy, alopecia, diabetes or neurotoxicity.

Sirolimus is metabolised by cytochrome P450 3A4 isoenzymes (see Panel 1). It must be taken at least four hours after ciclosporin when the two are used together because ciclosporin markedly increases the bioavailability of sirolimus when they are co-administered.

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**INHIBITING IL-2 PRODUCTION**

Ciclosporin enabled graft survival rates to increase to a level where many local transplant programmes became viable. It is licensed for all solid organ grafting and shares a common mechanism with tacrolimus. Tacrolimus is a newer drug and is licensed for use in liver and kidney transplant regimens.

Once bound to their respective cellular proteins both drugs will prevent newly activated T cells from transcribing the genes that code for interleukin-2. Both drug-immunophilin complexes target the enzyme calcineurin (a rate limiting point in the early T-cell activation cascade) and are genetically termed calcineurin inhibitors (CNI). Preventing cytokine production halts the T-cell driven immune response. The two drugs are alternatives and are not used in combination.

Neoral is the only licensed brand of ciclosporin currently available in the UK although some patients remain on Sandimmun which is still available on a named-patient basis from Novartis. The increased and more reliable absorption seen with the newer, pre-emulsion formulation means that the two are not interchangeable without close supervision. The BNF recommends that the brand be specified on all prescriptions. A normal, initial oral dose will be 4 or 5mg/kg given every 12 hours. Thereafter doses are adjusted by whole blood levels (ciclosporin and tacrolimus are bound extensively to erythrocytes). Historically, trough levels have been used, typically aiming initially for 150-350ng/ml. This target will be lowered after the early, high rejection risk period, usually to around 75-150ng/ml. Some units are now taking a two hour, post-dose level (called C2 monitoring) which is a better surrogate of exposure to the drug. Achieving target C2 levels has been demonstrated to result in greater probability of freedom from rejection in both kidney and liver recipients. Unfortunately, the practicalities of C2 monitoring, particularly in outpatient settings, limit the potential of what is clearly a better method of therapeu-
tic drug monitoring.

The more potent tacrolimus is usually started at around 0.1 to 0.15mg/kg every 12 hours. Only trough monitoring is undertaken. Typical desired initial levels are in the order of 10–15ng/ml. Tacrolimus has largely replaced initial use of ciclosporin in UK liver transplant immunosuppression following the “TMC” study and there is evidence that its use results in significantly fewer acute rejections compared with trough monitored Neoral in renal transplant patients.

Both these agents can be given intravenously if patients are nil by mouth. To take account of their relatively poor bioavailability the daily dose should be one-third of the oral daily dose. Ciclosporin is given as a twice daily, intermittent infusion and tacrolimus as a continuous infusion, avoiding incompatible PVC equipment.

Ciclosporin and tacrolimus share a number of side effects. The prevalence and severity of these differ between the two. Effects common to both include nephrotoxicity (seen acutely and as a chronic, largely irreversible effect), hyperlipidaemia, hypertension, glucose intolerance and neurotoxicity. Ciclosporin is associated with hirsutism and tacrolimus has caused alopecia. Gingival hyperplasia occurs with ciclosporin, particularly if patients also take nifedipine.

As with sirolimus, both ciclosporin and tacrolimus are metabolised by cytochrome P450 3A4 (see Panel 1, p203).

### IL-2R Antibodies

Until recently, the practice of induction treatment was limited by both the acute adverse effects and the longer term risks of excessive immunosuppression seen with the anti-lymphocyte globulins or OKT3. Basiliximab and daclizumab are monoclonal human/mouse antibodies manipulated to disguise the mouse region and so prevent antibody formation in the recipient. Both are licensed for renal transplant and are initially given pre-operatively as a short infusion. The dose of basiliximab is repeated once on day 4 and daclizumab is given fortnightly a further four times. The intention with both is that they exert their immunosuppressant action only over the first few crucial weeks post-transplant.

Both antibodies are directed to bind specifically to that part of the IL-2R found only on the surface of activated lymphocytes. Once bound, the antibodies reduce the sensitivity of the receptor to IL-2, preventing the clonal expansion of those T-cells already stimulated by antigen. Unlike the older antibodies (ATG or OKT3) neither has an effect on the recipient’s population of resting T cells.

Basiliximab has been demonstrated to reduce the incidence of acute renal transplant rejection by around 30 per cent six months after grafting when compared with placebo. Likewise, daclizumab reduced acute rejection rates from 35 per cent to 22 per cent in a similar study. The incidence of adverse effects, early malignancies and infectious complications were comparable in the placebo and drug arms of these studies.

### Combination Treatment

Immunosuppressants have been used in combination since the first transplants. The aim is to minimise the doses of drugs with a low therapeutic index (and, where possible, “spare” steroids) while reducing the prospect of acute rejection.

Until the introduction of ciclosporin in 1982 most renal transplant regimens used prednisolone with azathioprine in their maintenance regimen. Adding ciclosporin, known to be non-myelosuppressive, was a logical next step. Only later was it discovered where in the sequence of the immune cascade this adjuvant was working. Our understanding of the pharmacology of these drugs now allows us to choose complementary agents. Triple therapy in one form or another is still the initial basis of most regimens. In many cases azathioprine has been replaced with mycophenolate or ciclosporin with tacrolimus.

The major advance over the past few years has been the use of initial regimens tailored more to the needs of a recipient than previous rigid, centre-specific protocols. For example:

1. Patients with known risk factors for a higher incidence of acute rejection (eg, retransplants) might be prescribed mycophenolate rather than azathioprine.
2. The early avoidance of nephrotoxic drugs might be beneficial in kidney recipients where donor factors (eg, prolonged organ cold ischaemia time) indicate a likelihood of delayed graft function. In such cases offsetting the loss...
of a CNI by using an IL-2R antibody with mycophenolate and steroids would make sense.

Most centres will now see markedly fewer rejection episodes (and, more importantly, fewer treatment-resistant episodes) as a result of this approach. Where rejection does occur there is an understanding that changing the CNI component (from cyclosporin to tacrolimus or vice versa) will augment first-line treatment with pulsed, intravenous steroids. Both cyclosporin and tacrolimus are licensed for this use.

Having a greater number of agents has also increased our ability to respond to potential adverse effects. For example:

- Cyclosporin may be replaced with tacrolimus where hirsutism is a debilitating cosmetic effect.
- Sirolimus, which has the potential to delay wound healing, might not be used de novo but might replace nephrotoxic ciclosporin in a patient who has suboptimal renal function.
- Using a slightly more potent maintenance immunosuppression might allow the withdrawal of steroids.

The key with all of these developments is to maintain the balance of immunosuppression. Long-term immunosuppression increases the risks of developing some types of cancer. Infection remains one of the most frequent adverse effects seen in immunosuppressed patients. One of the “fathers” of transplant immunosuppression is quoted as referring to mercaptopurine as the “fathers” of transplant immunosuppression.

**CONCLUSION**

Recent developments in the way we use immunosuppressant drugs have been effective in reducing the incidence of early acute rejection. The key challenge now is to ensure this translates into reduced chronic rejection and longer graft life.

*Credit for Learning begins on p229*

**REFERENCES**