

Immunosuppression in kidney transplantation: A review and a personal approach

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■ ABSTRACT

This is a short review of the history of immunosuppression describing the principal characteristics, the mode of action and the side effects of each immunosuppressive agent currently used in clinical practice. It highlights the most commonly administered immunosuppressive therapy, the triple combination of MMF/Mycophenolic acid + Calcineurin Inhibitor + Steroids. It also emphasises the importance of tailoring treatment to the individual patient, especially in cases such as hypersensitised and black patients, retransplants and poor compatibilities, which have a higher risk of acute rejection. A review of the literature and our own experience have enabled us to construct a set of practical rules to help achieve the best possible immunosuppressive regime for each patient.

Key-Words:

Calcineurin inhibitors; immunosuppression; kidney transplantation; mTOR inhibitors; rejection; tolerance.

Organ transplantation has been one of the greatest achievements in medicine of the last century, as the United Network for Organ Sharing (UNOS) acknowledged. According to this major US and world transplantation organisation: “Organ transplantation was recognized as the third most gripping moment in medical history following Alexander Fleming’s discovery of Penicillin in 1928, and the invention of the Polio vaccine by Jonas Salk in 1955” (see its Update, February 2000).

■ INTRODUCTION

Organ or tissue transplantation between two individuals of the same species (homotransplantation) requires effective control of the recipient’s immunological system to prevent graft rejection. The first description of this reaction against foreign tissue is attributed to Tagliacozzi¹ who, in the 16th century tried to repair damaged noses by performing surgical grafts with skin obtained from another individual. These grafts were immediately rejected, so Tagliacozzi concluded that: “*the singular nature of the individual totally dissuaded us from trying this on another person*”. When Joseph Murray² performed the first successful kidney transplantation in 1954 there was no immunosuppression (IMS), and this success was only possible because the transplant was performed between two identical twins, that is to say, two immunologically identical beings. Murray was aware of this rare opportunity, a gift from nature, leading him to say: “*we did not solve the problem, we bypassed it*”. Sir MacFarlane Burnet³, who was awarded the Nobel Prize for Medicine in 1960, drew attention to the important issue of the reaction of the immune system and pointed out that: “*...much thought has been given to ways by which tissues or organs not genetically and antigenetically identical with the patient might be made to survive and function in the alien environment. On the whole, the present outlook is highly unfavourable to success...*”. Sir Peter Medawar⁴, who shared the Nobel Prize with MacFarlane Burnet in

1960, was the first to describe the immunological nature of rejection as being mediated by “*immuno-competent cells*” – the lymphocytes. Murray had referred to this system and its defence mechanisms when he discussed “the problem”: without any manipulation, the organisms do not accept something they identify as foreign.

For successful transplantation it is necessary to create in the recipient conditions conducive to receiving the graft and permitting its normal functioning. The initial attempts to control the immunological response and to make the graft “accepted” by the recipient were carried out using a physical process – total irradiation of the recipient’s body with X-rays⁵. Meanwhile, in 1959, Schwartz and Damscheck⁶ discovered the immunosuppressive properties of 6-mercaptopurine (6-MP), a drug that works by inhibiting the clonal proliferation of lymphocytes. It was from 6-MP that azathioprine (AZA) was developed and immediately (1960) investigated by Roy Calne and Joseph Murray, who tested it on dogs receiving renal allografts⁷. AZA has an effect on both T and B lymphocytes as an anti-proliferative agent⁸. However, preliminary studies demonstrated that AZA alone did not seem to be sufficiently effective in preventing graft rejection. As a result, Starzl⁹ combined corticosteroids with AZA and performed a series of renal allografts using this combination. With this immunosuppressive scheme, Starzl obtained the best results produced in a clinical setting up to that time.

Over the following years, the AZA+corticosteroid combination was the only immunosuppression (IMS) used in transplantation until antilymphocyte serum emerged as a result of the research of Woodruff and Anderson¹⁰ who developed it from lymphocytes collected by thoracic duct drainage. Starzl *et al.*¹¹ demonstrated the antirejection properties of these new antilymphoid agents, designated polyclonal antibodies. At the beginning of the 1980s a new class of antibodies, monoclonal antibodies (*e.g.* OKT3)¹², emerged, offering potential advantages over polyclonal antibody preparations in being more specific and having fewer side effects.

The great revolution in the field of IMS occurred with the discovery of Cyclosporine A (CsA), a calcineurin inhibitor. The first studies of this new drug appeared at the end of the 1970s¹³, and from 1983 onwards it began to be used in the main transplantation centres and later became common throughout practically the

whole world. With this new immunosuppressive agent, the triple combination of AZA+CsA+corticosteroid became the most frequently used regimen.

Tacrolimus (FK506), another calcineurin inhibitor¹⁴, was first used in 1993 and began to compete with it.

Mycophenolate Mofetil (MMF) was introduced in 1995 and rapidly proved to be more effective than AZA in the prevention of acute rejection (AR)¹⁵. In 1998, the CD25 antibodies, basiliximab and daclizumab, started to be used in clinical practice as part of induction therapy¹⁶. In the same year, a new class of immunosuppressant, the mTOR (mammalian target of rapamycin) inhibitors appeared with rapamycin (Sirolimus), whose preliminary studies suggested that it had a comparable efficacy to the calcineurin inhibitors in the prevention of AR, but without their nephrotoxic effects¹⁷.

More recently, two new immunosuppressants have been introduced into clinical transplantation – mycophenolic acid (Myfortic), from the same group as MMF, and Everolimus, another mTOR inhibitor.

The progress that has been achieved so far has resulted in a significant reduction in the incidence of AR, which is now below 20% in most transplantation centres and in some cases below 10%. This has enabled graft survival to reach over 90% in the 1st year¹⁸. However, these remarkable improvements in rates of AR have not been mirrored in long-term graft survival, as shown by the cadaveric graft half-life which does not exceed 12 years¹⁹. Furthermore, the toxicity of IMS, mainly on the kidney and on the cardiovascular system, has produced harmful effects on the graft and on the patient²⁰. The main causes of graft loss are patient death with a functioning graft and chronic graft dysfunction, with the primary cause of patient death being due to cardiovascular disease²¹. Moreover, immunodeficiency, a consequence of more powerful IMS, has caused an increase in the incidence of infections and malignancy²².

Research into IMS aims to find a therapy that can induce tolerance without these restrictions and drawbacks. The ideal immunosuppressant would be one that could induce total tolerance avoiding rejection (acute or chronic), without any relevant side effects and with economically acceptable costs.

■ GENERAL CONCEPTS

Ideally, IMS should be specific, targeted at the organ that is to be protected. This is not possible at the moment, and therefore IMS affects the whole organism with three main types of effects:

- It suppresses the host's response and encourages tolerance to the graft, which is the desired effect;
- It has a non-immunological toxicity (nephrotoxicity, diabetes, hyperlipidaemia, arterial hypertension) which is potentially very harmful to the patient;
- It causes immunodeficiency which can have serious consequences (infections, malignant disease).

The toxicity and immunodeficiency induced by IMS are a cause of significant morbidity and mortality.

There are three types of IMS: induction, anti-rejection (reversal of rejection) and maintenance.

■ IMMUNOSUPPRESSIVE AGENTS

■ Ciclosporin

Cyclosporine A (CsA) was originally obtained from the fermentation products of the fungal species *tolypocladium inflatum*.

CsA entry into the T cell, binding to cyclophilins and this complex ciclosporin-cyclophilin-calcineurin inhibits the phosphatase activity of calcineurin and through this mechanism it then inhibits the synthesis of IL-2 and the receptors of IL-2, IL-4 and gamma interferon by the T lymphocytes, thereby blocking the transcription of the genes of these cytokines²³. It does this because it dephosphorylates the cytosolic form of nuclear factor of activated T cells (NFAT), which is necessary for its translocation into the nucleus and subsequent promotion of IL-2 gene transcription. CsA also increases the expression of TGF- β which may contribute to its immunosuppressive properties inhibiting T cell growth and activation, but also to its adverse effects, including renal fibrosis.

The appearance of CsA as an immunosuppressant represented such a revolution in the transplantation

world that transplant IMS has been divided into two eras: pre-ciclosporin (AZA+steroids) and post-ciclosporin. The dramatic improvements observed in the results, namely in 1-year patient and graft survival, has made transplantation a much safer and more efficacious therapy. In kidney transplantation, the global rate of AR went down from 80% to 50%²⁴, and graft survival in the 1st year went up from 60% to 90%²⁵. However, it soon became understood that CsA was nephrotoxic. As Roy Calne wrote in 1978: *"Cyclosporine was effective in inhibiting rejection but there was clear evidence of both nephrotoxicity and hepatotoxicity"*²⁶.

This toxicity of CsA is one of the factors that contributes to chronic allograft nephropathy which is now the main cause of graft loss. Furthermore, CsA also has a persistent vascular toxicity with a generalised vasculopathy, causing arterial hypertension and cardiovascular morbidity²⁷. Other side effects associated with CsA include hyperglycaemia, hypertrichosis, gingival hyperthrophy and malignancy (mainly an increased incidence of lymphomas and skin cancers).

Despite its toxicity, the gold standard for IMS from 1983 to 1996 was the combination of CsA with low-doses of azathioprine and prednisone, and this regimen was used by more than 90% of centres all over Europe.

Most of the drug is excreted in the bile and only 6% through urine. It is also eliminated through breast milk so it is not recommended for those who are breastfeeding. It can be monitored in the blood, making it possible to adjust the doses according to the required concentrations. The initial recommended dosage is around 8mg/Kg/day taken in 2 doses in order to reach Co (lowest blood concentration) between 150-300 ng/ml, and C₂ (blood concentration two hours after medication) between 1500-2000ng/ml. After the first two months, the recommended Co levels vary between 100-200 ng/ml.

■ Tacrolimus (FK506)

Tacrolimus (Tac) is a macrolide which is derived from *Streptomyces Tsukubaensis*. Like CsA, it blocks the transcription of the IL-2 gene in T lymphocytes. Tac binds to a protein that is denominated FKBP ("the FK

binding protein”) and the complex inhibits calcineurin²³. Experimental studies have shown that Tac is more powerful than CsA (about one hundred times more) as it inhibits the formation of cytotoxic T cells in the Go-G1 phase of the cellular cycle²⁸. The inhibition of the renal calcineurin phosphatase explains the nephrotoxicity of both CsA and Tac. Its excretion is essentially in the bile and only 5% is through the kidney.

Side effects include neurotoxicity, nephrotoxicity and hyperglycaemia (diabetes). Like CsA, the dosage of Tac is adjusted to blood concentrations. The initial dose is usually 0.10 to 0.20 mg/Kg/per day, divided into two doses, and the desired blood level concentrations (Co) should be 10-15 ng/ml early after transplantation and 5-10 ng/ml later.

■ Sirolimus/ Everolimus

These are both mTOR inhibitors (*mammalian target of rapamycin*).

Rapamycin (Sirolimus – SRL) is a macrolide produced by *Streptomyces hygroscopicus*, a powerful immunosuppressant that penetrates the plasma membrane and binds to FKBP-12 immunophilin, forming a complex that inhibits the enzymatic activation of mTOR and thereby controlling the action of various enzymes which prevent the progression of the cellular cycle from the G1 phase to the S phase in various cell lines²⁹. Thus, SRL blocks the growth factor that mediates the proliferation of T and B cells and vascular smooth muscle cells through 2 pathways; one calcium-dependent and the other calcium-independent³⁰. The immunosuppressive effect of SRL is the result of three main mechanisms³¹:

- 1) Anti-proliferative effect (on the lymphoid cells);
- 2) effect on co-stimulation (it inhibits the stimulation of CD28);
- 3) blocking of protein synthesis.

SRL and everolimus are administered orally and are absorbed mainly through the small intestine. In contrast with the CNIs (CsA and Tac), SRL has been shown to be non-nephrotoxic in both animal and human experiments³². Several clinical trials have shown that SRL is well-tolerated, efficacious in the prevention of AR, enables good graft and patient

survival in the 1st year, gives good renal function and also has anti-proliferative properties³³. In studies investigating early withdrawal of CsA from a regimen with SRL and steroids, better renal function and histology were found at three years in the group without CsA³⁴. Its main side effects are hyperlipidaemia, myelotoxicity, pneumonia and delay in surgical wound healing. The occurrence of any of these side effects may require a reduction in drug dosage or even drug withdrawal. The initial dosage of SRL varies between 2-6 mg/day with the aim of achieving blood concentrations of 5-15 ng/ml initially (later 5-10 ng/ml). Everolimus is administered in an initial dosage of 1.5-3 mg/day aiming for blood concentrations of 3-8 ng/ml.

■ Mycophenolate Mofetil (MMF)

MMF is an ethyl ester of mycophenolic acid (MPA) produced by *Penicillium glaucum*. It has very good oral bio-availability and after its absorption the ester is hydrolysed releasing MPA, which inhibits the enzyme inosine monophosphate dehydrogenase (IMPDH) in *de novo* purine metabolism. The anti-proliferative activity of MPA is particularly effective at inhibiting T and B lymphocyte proliferation and, at the same time, glycosylation of adhesion molecules adds to the anti-rejection action. Experimental data suggest that MMF can inhibit the endothelial synthesis of nitric oxide which is mediated by cytokines and can also reduce myointimal proliferation in the allograft. As a result, MMF is capable of diminishing the severity of the histological lesion of chronic rejection²³.

Side effects include gastro-intestinal disorders (abdominal pain, nausea, vomiting, diarrhoea), leucopaenia, thrombocytopaenia and anaemia.

The recommended dosage is 1000 mg twice a day (2000 mg/day).

■ Mycophenolic Acid (Myfortic)

More recently mycophenolate sodium with an enteric coating has appeared as an alternative to MMF with the aim of reducing or obviating the gastro-intestinal side effects of MMF. Myfortic is available in capsules of 360mg and the recommended dose is 720 mg every 12 hours with a total dose of 1440 mg/day.

■ Corticosteroids

Corticosteroids have a powerful anti-inflammatory action that reduces the infiltration of macrophages at the inflammatory sites³⁵. Their action is mainly in the earliest phase of the immune reaction, at the level of the macrophages and T lymphocytes, and they have little influence on the production of antibodies. They inhibit the synthesis of IL-1, IL-6 and IFN- γ by blocking the transcription of their respective genes. The inhibition of IL-1 inhibits, in turn, IL-2 and the result is a blocking of the initial phase of lymphocyte activation²³.

Side effects are manifold: obesity, diabetes, Cushing syndrome, arterial hypertension, slow growth in children, peptic ulcer, psychosis, depression, bone aseptic necrosis, osteoporosis, cataracts, and acute supra-renal insufficiency.

Due to the side effects of these corticosteroids, they should be used sparingly or even avoided in kidney transplant patients. The danger of an IMS without corticosteroids is the increased risk of AR and/or graft loss, as the metanalysis by Kasiske *et al.*³⁶ has shown. Therefore, transplant centres have tried to develop immunosuppressive regimens which seek to progressively decrease the dose until they can be withdrawn at three months. Another strategy that has been proved to be successful is the development of steroid-free maintenance regimens. Vincent *et al.*³⁷ and Tter Meulen *et al.*³⁸ did IMS with anti IL-2 receptor, MMF and CNl, withdrawing the corticosteroids before the end of the first week without increasing the incidence of AR. In these cases we must perform induction therapy with monoclonal or polyclonal antibodies.

■ Polyclonal Antibodies

These are used in induction therapy and in anti-rejection therapy.

1) Thymoglobulin

This is a rabbit anti-human thymocyte immunoglobulin.

Its main immunosuppressive mechanism is exercised through the elimination of the lymphocytes, since it is capable of recognising the majority of

molecules involved in the activation cascade of the T lymphocytes during rejection of the graft³⁹. Dosage: induction therapy – 1.25 to 2.5mg/kg/day for 3 to 10 days; and in anti-rejection therapy – 2.5 to 5mg/kg/day for about 5 to 7 days.

Main side effects are fever, chills, hypotension and tachycardia. The most serious effects are bone marrow suppression (leucopaenia and trombocytopenia) and a greater susceptibility to infection (particularly CMV).

2) ATG (Fresenius)

This is a rabbit anti-T-lymphocyte serum that works by the same mechanism as thymoglobulin, with a similar side effect profile.

Dosage: induction therapy – 0.1 to 0.15 ml/kg/day for 5 to 14 days; anti-rejection therapy – 0.15 to 0.25 ml/kg/day for 5-14 days.

■ Monoclonal Antibodies – IL2 anti-receptors

Basiliximab

This is a chimeric anti-CD25 monoclonal antibody (human/murine). By joining itself to the α -chain of the IL-2R (IL-2 receptor) it competes with IL-2 inhibiting the proliferative response. It prevents the activation and differentiation of T lymphocytes that are mediated by IL-2, which are fundamental for the occurrence of rejection.

Dosage – 20 mg on days 1 and 4.

Daclizumab

This is also an anti-CD25 monoclonal antibody, but humanised with a longer action than Basiliximab, and it works in a similar way. Dosage – 25 mg a week for 4 weeks.

Very few or no side effects are reported in studies of these two monoclonal antibodies.

■ Anti-CD3 Monoclonal antibody (Orthoclone – OKT3)

This is a murine monoclonal antibody directed against the CD3 antigen. It causes depletion of CD3+ T lymphocytes by cellular lysis.

Side effects: fever, chills, headaches, vomiting and myalgias. In 5% of cases there is acute pulmonary oedema due to a combination of volume overload, the increase in pulmonary vascular permeability and decrease in ventricular contractility³⁹.

Dosage – 2.5-5 mg/day i.v. *bolus*, for 7 to 14 days.

■ TOLERANCE

This is defined as the maintenance of graft survival over time without IMS. This tolerance could be achieved from at the time of transplantation through, for example, microchimerism (the presence in the recipient of a small number of cells from the bone marrow of the donor), gene therapy or lymphocyte depletion⁴⁰. Tolerance can also be induced by IMS after variable periods of time. Despite the progress that has been made in IMS some difficulties persist. The induction of immunological tolerance through the intensive manipulation of the immunity of the recipient during the first weeks immediately after transplantation still remains the primary objective.

■ THE IMS REGIMEN

In the majority of the transplantation centres triple therapy regimens are practiced: MMF/ Myf (in some cases AZA), Calcineurin Inhibitors (CsA, Tac) and steroids. In our experience these regimens have produced the lowest rate of AR which is one of the principal objectives to reach, knowing that acute rejection is the main risk factor for chronic rejection. However, CNIs are nephrotoxic and this contributes to graft loss. The toxicity of CNIs justifies consideration of other combinations of immunosuppressants in order to obtain similar efficacy with less toxicity. Consequently, the mTOR inhibitors, which are not nephrotoxic, can be an alternative to the CNIs. However, some controversial results⁴¹, and the early side effects that interfere with surgical recovery and graft function, as well as the short follow-up period at the moment, mean that prudence is required in considering them as first line therapy. So regimens with CNIs as first line therapy continue to be the gold standard in IMS in kidney transplantation.

■ INDIVIDUALISATION OF IMMUNOSUPPRESSIVE REGIMENS

The following four topics help us to tailor IMS and its follow-up:

- molecular or cellular monitoring
- biochemical levels
- clinical evaluation
- computer programs

The individualisation of IMS is particularly important in some specific situations including⁴²:

- 1 – Two haplotypes versus all other types
- 2 – Living donor versus cadaveric donor
- 3 – Young people versus old people
- 4 – Black race versus other races
- 5 – Good compatibility versus poor compatibility
- 6 – Delayed graft function (ATN) versus immediate functioning
- 7 – First transplant versus second or third transplant
- 8 – A high PRA versus a low PRA

■ THE MAIN FACTORS TO CONSIDER IN THE CHOICE OF IMS⁴³:

Factors	Variables
Donor	Living, cadaver, cause of death, age, good, weak, marginal
Organ	Kidney size (match donor-recipient), cold ischaemia time
Recipient	HLA mismatches, race, age, initial dysfunction
Immunosuppression	Efficacy, toxicity (myelosuppression, dyslipidaemia)
Co-morbidities	Obesity, liver pathology, cardiovascular pathology, latent infections
Immunological risk	Sensitisation and HLA mismatches, retransplants, Black race, cold ischaemia time, blood transfusions, previous pregnancies.

■ SOME RULES FOR THE DRAWING UP/SELECTION OF IMS PROTOCOLS

1st The most common triple combination is MMF/ Myf + CNI + steroids.

2nd In a combination MMF/Myf + mTORIn + steroids, without CNI, induction therapy with polyclonal or monoclonal antibody is recommended.

3rd The use of mTORIn is not recommended as an initial therapy in patients with wound healing problems (obese, diabetic) and/or with initial graft dysfunction.

4th The combination mTORIn + CNI + steroids has been mostly used with CsA and the possibility of early withdrawal (at 3 months) of the CNI should be considered.

5th The replacement of the CNI and its substitution by an mTORIn, should be made early (before or at least from the 3rd month) in order to reduce exposure to the CNI. Also, the conversion to an mTORIn, when signs of chronic dysfunction appear, should only be considered if the serum creatinine is less than 2.5mg/ml and proteinuria is less than 800 mg/24h. If serum creatinine is higher than 2.5 ml/min, GFR less than 40ml/min, and proteinuria higher than 800 mg/24hr, then conversion is not recommended.

6th In patients with a high immunological risk (PRA >50%, black race, retransplants, extremely urgent condition without compatibility, children) an induction therapy, preferably with polyclonal antibody, should be done and the triple combination indicated in rule 1, with Tac, should be used.

7th In patients who have risk factors for diabetes, tacrolimus should be avoided and steroids should be withdrawn as soon as possible.

8th The withdrawal of steroids as soon as possible because of their toxicity is one of the aims of any IMS regime. However, in high risk patients, this strategy must be considered on the basis of individual patient evaluation.

9th In patients with low immunological risk the combination of monoclonal or polyclonal antibody + MMF/Myf + CNIs + steroids allows for the safe stopping of steroids after the 3 initial *bolus* doses of methylprednisolone (day 0 – 500mg, day 2 –250mg, day 4 – 125mg) or at day 7.

10th Monotherapy with Tac after the first six to 12 months, in which the withdrawal of steroids is followed by the withdrawal of MMF/Myf, is a possibility worth considering.

11th Transplant patients with a history of cancer or/and those that after transplantation develop a malignancy, must be switched as soon as possible from the CNI to an mTORIn, whose antiproliferative properties could have a beneficial effect on cancer control.

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