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REVIEW

Immunosuppressive drugs after solid organ transplantation

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ABSTRACT

In recent years solid organ transplantation has been rapidly developed as a therapeutic intervention that is life-saving and greatly contributes to a better quality of life in organ recipients. The rapid development has been made possible because of a drastic expansion in the immunosuppressive repertoire. Unfortunately, the side effects of these drugs can be severe, which is one of the reasons that life expectancy of transplant patients still significantly falls short of that of the general population. In this review manuscript we will discuss current and future immunosuppressive strategies that are employed in solid organ transplantation. Expanding our understanding of the human immune system will hopefully provide us with newer, smarter drugs that promote immunotolerance without the side effects observed today.

KEYWORDS

Solid organ transplantation, renal transplantation, immunosuppressive drugs

INTRODUCTION

In 1954, the first successful renal transplantation was performed at the Peter Bent Brigham Hospital in Boston.[1] Because the donor and recipient were identical twins, there was no need for immunosuppression. This success underlined the surgical feasibility of organ transplantation and greatly stimulated research into immunosuppression, opening up the possibility to extend transplantation beyond identical twins.

In the 1950s, sublethal doses of total body irradiation (TBI) were combined with cortisone.[2] Although TBI did produce adequate immune suppression, it also resulted in profound bone marrow aplasia, which often led to patients dying from overwhelming infections.

The breakthrough came in 1959, when it was reported that 6-mercaptopurine (6-MP), which was already in use for acute lymphocytic leukaemia, suppressed the immune system.[3,4] Soon, the first clinical trial using a combination of corticosteroids and 6-MP was set up. It delivered one-year rates of allograft survival in the range of 40-50%.[5] A few years later, 6-MP was replaced by its prodrug azathioprine, which was equally effective but less toxic. Also, antithymocyte globulin (ATG) was introduced: first to treat corticosteroid-resistant rejection episodes and later as part of induction protocols. Several trials followed, producing rates of one-year graft survival of around 70%.[6-8]

In the early 1980s, the introduction of cyclosporine marked a new era in clinical transplantation, increasing one-year graft survival rates to well over 80%.[2]

In the last 20 years, the immunosuppressive repertoire has been extended significantly with the introduction of drugs, such as tacrolimus, mycophenolate mofetil and sirolimus, and monoclonal antibodies, such as basiliximab and alemtuzumab. These drugs have facilitated major improvements, especially in one-year graft survival, which now exceeds 90% in most centres. Unfortunately, long-term graft survival still lags behind, with only a very modest increase compared with the early days of transplantation medicine.

Here, we present an overview of the drugs currently used for immunosuppression after solid organ transplantation. Our focus is mainly on renal transplantation, but the general principles apply for all types of organ transplantation. We will discuss mechanisms of action, major toxicities and the place in the immunosuppressive regimen for these drugs. *Tables 1* and *2* provide a summary.

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Table 1. *Overview of drugs currently used in solid organ transplantation*

Drug	Trade name	Place in treatment protocol	Comments
Glucocorticosteroids		Induction and maintenance; acute cellular rejection and AMR	Role in maintenance immunosuppression under investigation because of severe side effects during long-term use
Azathioprine	Imuran	Maintenance	Mainstay of immunosuppression together with glucocorticosteroids until 1980s, producing one-year graft survival of around 70%
MMF mycophenolic acid	CellCept Myfortic	Maintenance	MMF, introduced in early 1990s, was initially favoured over azathioprine, but newer trials show similar efficacy
Calcineurin inhibitors (Cyclosporine/ Tacrolimus)	Neoral Prograft/ Advagraf	Maintenance	CNIs were introduced in the 1980s-1990s and revolutionised maintenance immunosuppression. Tacrolimus has a lower risk of acute rejection and allograft loss than cyclosporin. The use of CNIs is limited by their side effects, especially nephrotoxicity
mTOR-inhibitors (Sirolimus/Everolimus)	Certican Rapamune	Maintenance	Place in maintenance immunosuppression still under investigation; often used to limit CNI nephrotoxicity
ATG		Induction; steroid-resistant rejection	Oldest available medication for induction and rejection treatment (apart from steroids), still highly effective but toxic
Alemtuzumab	MabCampath	Induction; steroid-resistant rejection	Place in induction and acute rejection treatment is still under investigation; appears to be similar to ATG while less toxic
Rituximab	MabThera	AMR, HLA-sensitised patients, ABO-incompatible transplantations	Has also been evaluated as induction agent, but unsuccessfully
Basiliximab	Simulect	Induction	Higher rejection rates one year post-transplantation than ATG, but less toxic
Belatacept	Nulojix	Maintenance	Promising new agent for maintenance immunosuppression, further studies are needed
Bortezomib	Velcade	AMR	Small, non-randomised trials suggest efficacy in AMR
Eculizumab	Soliris	AMR	May decrease AMR in highly sensitised individuals

AMR = antibody-mediated rejection; ATG = antithymocyte globulin; CNI = calcineurin inhibitor; MMF = mycophenolate mofetil; mTOR = mammalian target of rapamycin.

Table 2. *Major toxicities of drugs currently used in solid organ transplantation*

Drug	Cardiovascular toxicity			Malignancies	Selected infections			Bone marrow suppression	Other
	Hypertension	Dyslipidaemia	DM		CMV[§]	EBV[#]	BKV[†]		
Glucocorticosteroids	++	+	++	-*				N	Cushingoid appearance, sleep disturbances, mood changes, impaired wound healing, osteoporosis
Azathioprine	N	N	N	+[$]				+	Hepatotoxicity
MMF	N	+	+	N		-	+	+	Gastrointestinal symptoms
Cyclosporine	++	+++	+	+				+	Nephrotoxic, neurotoxic, gum hyperplasia
Tacrolimus	+	++	++	+			+	+	Nephrotoxic, neurotoxic, gum hyperplasia
mTOR-inhibitors	N	+++	+	-	-			+	Impaired wound healing, flulike syndrome, acne
ATG				+	+	+		++	Cytokine-release syndrome
Alemtuzumab				-		+		+	Mild cytokine-release syndrome, induction of autoimmune disease
Rituximab				-		-		+	Infusion reactions
Basiliximab				-		+		+	Hypersensitivity reactions
Belatacept	++	++	+	+				+	
Bortezomib				-				+	Neurotoxicity
Eculizumab								+	Very expensive

***Used in many treatment protocols for malignancies; however, an increased risk of malignancy has also been described; [$]historically associated with an increased risk of malignancy, when azathioprine was given in high dosages. The risk association for currently used lower dosages is less clear; [§]CMV infections are increased with all immunosuppressive medications; however mTOR-inhibitors are thought to decrease the risk, whereas ATG increases the risk; #EBV increases the risk of PTLD (post-transplant lymphoproliferative disorder). Induction or rejection therapy with polyclonal and monoclonal antibodies increases the risk of PTLD. PTLD can be treated with rituximab; [†]The risk of a BKV infection is mostly dependent on the total load of immunosuppression, but MMF and tacrolimus appear to increase the risk.**
ATG = antithymocyte globulin; BKV = BK virus; CMV = cytomegalovirus; DM = diabetes mellitus; EBV = Epstein-Barr virus; MMF = mycophenolate mofetil; mTOR = mammalian target of rapamycin.

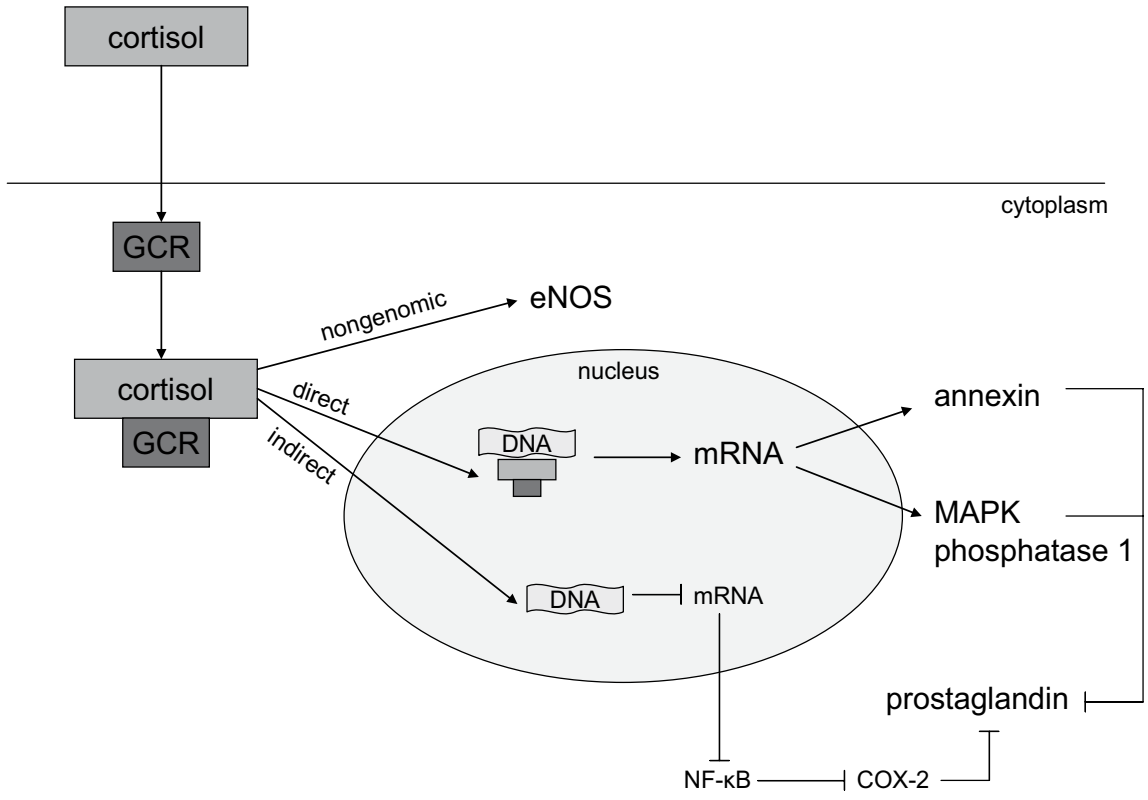
GLUCOCORTICOSTEROIDS

Since their discovery just after World War II, glucocorticosteroids have become one of the most widely used drugs in modern medicine. Glucocorticosteroids inhibit inflammation through three mechanisms: direct genomic effects, indirect genomic effects and nongenomic mechanisms,[9] illustrated in *figure 1*. Direct genomic effects occur when the cortisol-glucocorticosteroid receptor complex moves to the nucleus and affects transcription. Two important examples are the induction of annexin 1 and MAPK phosphatase 1. Each of them inhibits prostaglandin synthesis, which in turn inhibits inflammation. Indirect genomic effects take place when the glucocorticoid-receptor complex interacts with other transcription factors. NFκB is inhibited through this mechanism, leading to a decrease of COX-2, which also inhibits prostaglandin synthesis. Nongenomic effects, i.e. effects not mediated by changes in gene expression and transcription, may explain why glucocorticosteroids can also act very rapidly. The best-described non-genomic mechanism involves the activation of endothelial nitric oxide synthethase (eNOS), which appears to protect against ischaemia and reperfusion-induced injury in mice.[10]

The net result of these pathways is a neutrophilic leukocytosis, accompanied by dramatic transient reductions in circulating eosinophils, monocytes, and lymphocytes.[11] Circulating T cells rapidly decline, due to a combination of effects, including redistribution,[12] inhibition of pro-inflammatory cytokines[9] and induction of apoptosis.[13,14] B cells are less affected and antibody production is largely preserved.[15]

Because of their wide scope of immunosuppressive effects, glucocorticosteroids are used both for induction and maintenance immunosuppression and for treatment of acute rejection episodes. However, there are several well-known side effects. In addition to opportunistic infections, these include a Cushingoid appearance, sleep disturbances, mood changes, hyperglycaemia, hypertension, alterations in lipid metabolism, impaired wound healing and osteoporosis. Therefore, the role of corticosteroids, especially their long-term use in maintenance immunosuppression, is a subject of active research. In a recent systematic review of 29 randomised controlled trials, steroid avoidance and steroid withdrawal strategies in renal transplantation were not associated with increased mortality or graft loss despite an increase in acute rejection episodes. However, follow-up was

Figure 1. *Mechanism of action of glucocorticosteroids*



COX-2 = cyclo-oxygenase 2; eNOS = endothelial nitric oxide synthethase; GCR = glucocorticosteroid receptor; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor kappa-B.

limited, varying from six months to five years.[16] Another meta-analysis[17] also concluded that acute rejections were increased in steroid avoidance protocols, without affecting graft or patient survival.

ANTIMETABOLITES

Antimetabolites are purine and/or pyrimidine inhibitors, blocking DNA synthesis. Two well-known examples are azathioprine and mycophenolate mofetil, which are both used for maintenance immunosuppressive treatment. Azathioprine (Imuran®) was among the first drugs to be used in solid organ transplantation. It is metabolised to 6-MP, which interferes with DNA synthesis. Its immunosuppressive action *in vivo* seems to be mediated mainly by its inflammatory properties.[18,19] However, it is also thought to stimulate T-cell apoptosis.[20] *Figure 2* summarises the mechanism of action of azathioprine and other immunosuppressive drugs.[21-25]

The main side effects of azathioprine are bone marrow suppression and hepatotoxicity. Historically, its use has also been associated with malignancies. However, whereas older data show a clear correlation, newer data analysing combined immunosuppressive medications in which azathioprine is generally used in lower dosages, are less clear-cut.[26]

Mycophenolate mofetil (MMF, CellCept®) was first used in the early 1990s.[27] MMF is a prodrug that is rapidly metabolised to its active metabolite mycophenolic acid. A few years ago, mycophenolic acid also became available directly as Myfortic®. MMF inhibits lymphocyte function by blocking purine biosynthesis via inhibition of the enzyme inosine monophosphate dehydrogenase.[28] In most eukaryotic cells, blocking inosine monophosphate dehydrogenase has little effect on cell division because purines can also be generated from nucleotide breakdown products, the so-called purine salvage pathway. Because B and T lymphocytes lack this pathway, MMF is a more selective antiproliferative agent than azathioprine.[29,30] Because of this, and because MMF is less hepatotoxic and is not associated with malignancies,[26] it was expected to replace azathioprine in the immunosuppressive repertoire, especially when several studies found that acute rejection rates for prednisolone/MMF/cyclosporine were lower than for prednisolone/azathioprine/cyclosporine.[31,32] However, all these studies used older preparations of cyclosporine. Studies using the newer micro-emulsification formulation (Neoral®) have shown similar efficacy and adverse effects for the two regimens.[33,34]

When deciding between azathioprine and MMF, several additional factors come into play. For example, azathioprine should be used with caution in patients treated with allopurinol, because this drug inhibits xanthine oxidase, resulting in an accumulation of active azathioprine metabolites.[35] MMF, on the other hand, causes more dyslipidaemia and diabetes mellitus and is associated with an increased risk for BK nephropathy.[36]

CALCINEURIN INHIBITORS

Cyclosporine (Neoral®) and tacrolimus (Prograf®, Advagraf®) are calcineurin inhibitors (CNIs). Cyclosporine binds to cyclophilin, whereas tacrolimus binds to FK-binding protein. Both result in calcineurin inhibition, which in turn inhibits translocation and activation of nuclear factor of activated T cells (NFAT), leading to downregulation of IL-2, 3 and 4, TNF-alpha, CD40L, G-CSF, IFN-γ and others.[37]

The development of CNIs – first cyclosporine and later tacrolimus – revolutionised the treatment of solid organ transplant patients, significantly improving graft survival rates. Both have become cornerstones of maintenance immunosuppression. However, nephrotoxicity has proven to be a major problem. Two mechanisms are at play.[38] The first mechanism, endothelial injury leading to heightened mesangial cell contractility, is potentially reversible, but the second mechanism, interstitial fibrosis, which may occur as early as three months after transplantation, is irreversible.

Over the last two decades, tacrolimus has gradually become the more widely used CNI, because it is associated with a lower risk of acute rejection and allograft loss, as shown by several studies and confirmed by meta-analysis.[39,40] Toxicity profiles are similar: both increase the risk of malignancy; cyclosporine is associated with slightly higher rates of hypertension, hyperlipidaemia and gum hyperplasia, whereas tacrolimus has more prominent neurological side effects and more cases of drug-induced diabetes and BK nephropathy.[36]

mTOR INHIBITORS

mTOR stands for mammalian target of rapamycin. Inhibition of this target prevents the transduction of the signal initiated by binding of IL-2 to its IL-2 receptor. This signal targets the mTOR complex, which has a key role in the regulation of various processes in the cell affecting cell growth and division.[41] In addition to immunosuppressive properties, mTOR inhibitors also have antiproliferative properties and are used for oncological indications. Sirolimus and everolimus (an active metabolite of sirolimus) are the main drugs in this class. Major side effects include thrombocytopenia, hyperlipidaemia and impaired wound healing. Sirolimus monotherapy is generally not nephrotoxic, but in combination with CNIs, significant nephrotoxicity has been described, due to increased blood levels of CNIs.[42,43] CMV infections, on

the other hand, seem to occur less frequently with mTOR inhibitors.[44,45]

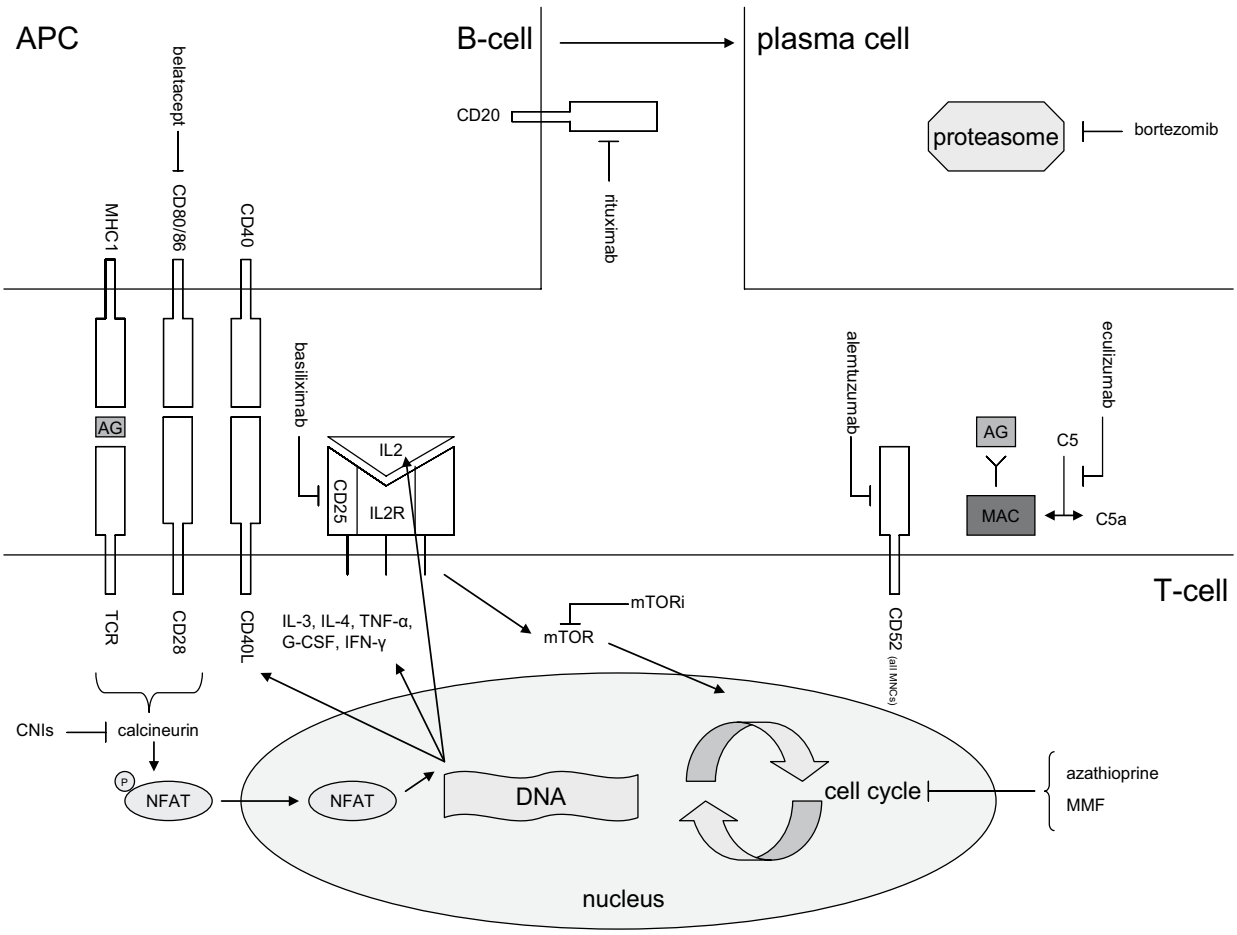
mTOR inhibitors are used for maintenance immunosuppression. A 2006 meta-analysis[46] comparing mTOR inhibitors with antimetabolites and calcineurin inhibitors concluded that mTOR inhibitors lowered the risk of acute rejection and a higher GFR. However, side effects were also more severe, particularly bone marrow suppression and lipid disturbances. The limitation of this meta-analysis was a follow-up of only two years. A randomised-controlled trial published in 2011[47] with a follow-up of eight years revealed different results: maintenance therapy with prednisolone/tacrolimus/MMF was accompanied by lower rates of acute rejection and a higher GFR than either prednisolone/tacrolimus/sirolimus or prednisolone/cyclosporine/sirolimus.

Overall, the place of mTOR inhibitors in immunosuppression after solid organ transplantation is still unclear. mTOR inhibitors are often used in patients experiencing CNI toxicity. A common approach is to start with a combined CNI-plus-mTOR inhibitor regimen, and aim for discontinuation of CNIs at three to six months post-transplantation, thereby avoiding irreversible CNI nephrotoxicity. Two trials for sirolimus[48,49] have shown that this results in improved renal function without significantly increasing acute rejection, whereas the recent ZEUS trial has shown that this holds true for everolimus as well.[50] Finally, in patients developing malignancies after transplantation (e.g. skin cancers, Kaposi sarcomas, post-transplantation lymphoproliferative disease (PTLD)), treatment with mTOR inhibitors seems to be a rational option.

DEPLETING ANTIBODIES

Drugs in this class include antithymocyte globulin (ATG), alemtuzumab and rituximab. ATG is a polyclonal

Figure 2. *Mechanism of action of selected immunosuppressive drugs*



AG = antigen; APC = antigen presenting cell (dendritic cell, macrophage or B-cell); CNIs = calcineurin inhibitors; G-CSF = granulocyte-colony stimulating factor; IL = interleukin; IL2R = IL-2 receptor; MAC = membrane attack complex; MHC = major histocompatibility complex; MMF = mycophenolate mofetil; MNCs = mononucleated cells; mTOR = mammalian target of rapamycin; mTORi = mTOR inhibitor; NFAT = nuclear factor of activated T-cells; TCR = T-cell receptor; TNF-α = tumor necrosis factor α.

immunoglobulin derived from either rabbits or horses that have been immunised with human thymocytes. In addition to T-cell depletion, it induces B-cell apoptosis, interferes with dendritic cell function, modulates adhesion molecules and chemokine receptors and induces regulatory T cells.[51] Administration of ATG induces a cytokine-release syndrome, which includes fever, chills and sometimes hypotension and pulmonary oedema. It also induces a profound lymphopenia that may last beyond one year.[52,53] ATG is used both as induction therapy and in the case of steroid-resistant rejection.

Alemtuzumab is a humanised monoclonal antibody against CD52. CD52 is present on T cells, B cells, NK cells and to a lesser extent on monocytes. As with ATG, alemtuzumab infusion can be followed by a cytokine-release syndrome, but this is much milder than with ATG.[54,55] Autoimmune phenomena have been observed, such as thyroid disease, haemolytic anaemia and thrombocytopenia.[56] Alemtuzumab can be used for induction therapy (see also next section). It is also under investigation for acute rejection. Preliminary studies[57-61] indicate that it may be an equally effective, but less toxic alternative to ATG in steroid-resistant rejection.

Rituximab is a monoclonal antibody against CD20, which is present on almost all B cells, except for plasma cells. In addition to being widely used in patients with haematological and rheumatoid disorders, rituximab is under study for application in antibody-mediated rejection (AMR),[62] desensitisation of HLA-sensitised patients[63] and ABO-incompatible transplantations.[64] It is also effective for PTLD.[65] Some studies have also evaluated rituximab as an induction agent, generally with disappointing results.[66-68] Side effects include infusion-related reactions.

NON-DEPLETING ANTIBODIES

The CD25 monoclonal antibody basiliximab is the main drug in this category. CD25 is the IL-2 receptor alpha chain on T cells and is expressed on activated T cells.[69,70] Side effects are relatively mild; hypersensitivity reactions have been described.[22]

Basiliximab can be used for induction therapy. Several studies have compared induction protocols using ATG, alemtuzumab or basiliximab. A Cochrane review[71] showed that basiliximab and ATG are equivalent in terms of graft loss or acute rejection at six months after transplantation, but that the use of ATG is accompanied by lower acute rejection rates at one year post-transplantation, at the cost of increased malignancies and CMV infections. A recent meta-analysis on alemtuzumab[72] showed that when compared with basiliximab, alemtuzumab results in fewer acute rejections. Alemtuzumab and ATG were equivalent in terms of acute rejection, graft loss, delayed graft function and mortality. Taken together, these results indicate that it is reasonable to reserve the use of ATG for high-risk patients, whereas basiliximab is a good option for low-risk patients, as several studies have shown.[73-75] The place of alemtuzumab in induction protocols remains to be settled, especially now that Genzyme Europe has withdrawn its marketing authorisation for commercial reasons.[76]

OTHER IMMUNOSUPPRESSIVE DRUGS

Belatacept, bortezomib and eculizumab are promising new candidates in this category. Belatacept is a fusion protein composed of the modified extracellular domain of CTLA4 and the Fc domain of human immunoglobulin IgG1. It blocks the CD80/86 co-stimulatory signal that is needed for T-cell activation, providing a new target for maintenance immunosuppression.[76] In low to moderate risk renal transplantation, short-term patient and allograft survival appear comparable with that observed under cyclosporine, with improved renal function despite more frequent and severe early acute rejection. Adverse effects include bone marrow suppression, hypertension, dyslipidaemia[36] and a relatively high frequency of PTLD.[77] Further research is needed to compare its efficacy and safety with other maintenance regimens.

Bortezomib, a proteasome inhibitor frequently used for treating multiple myeloma, is active against mature plasma cells. This sets it apart from many other immunosuppressive drugs, which can deplete immature B cells but not plasma cells. This makes bortezomib a promising candidate in the treatment of antibody-mediated rejection, traditionally associated with poor allograft survival. In one study comparing the addition of bortezomib or rituximab to a standard treatment protocol, 18-month graft survival in the bortezomib group was significantly higher.[78] Several other small studies[79] seem to confirm improved graft survival, especially in early AMR, but larger studies are needed. Neurotoxicity, headache, fatigue and bone marrow suppression are major side effects.

Eculizumab is a humanised monoclonal antibody that blocks the cleavage of human complement component C5 into its pro-inflammatory components.[80] This drug is available for the treatment of paroxysmal nocturnal haemoglobinuria (PNH) and atypical haemolytic uraemic syndrome (aHUS). Together with bortezomib and rituximab, it may be useful in the treatment of AMR. One study showed that eculizumab reduced the incidence of AMR in highly sensitised individuals when administered immediately post-transplantation.[81] A limitation is its price: for PNH, the yearly cost is estimated to be $ 400,000.

FUTURE DEVELOPMENTS

The ultimate goal of transplantation medicine is the induction of tolerance, which would eliminate the need for lifelong use of immunosuppressive medication. Indirect evidence that the human immune system possesses mechanisms to promote tolerance has been available for a long time as reflected by patients who have discontinued their immunosuppressive medication (e.g. due to noncompliance or for medical reasons such as persisting infections or malignancy), but still have functioning transplants.

Recent research has identified specific regulatory immune cells, which are specialised leukocyte populations that are either selected to have regulatory function during their development or acquire immunosuppressive properties in the local microenvironment of the allograft or in the graft-draining lymphoid tissues.[82] Regulatory T cells appear to play a central role, but regulatory B cells, macrophages, dendritic cells, myeloid-derived stromal cells and mesenchymal stromal cells also exist. A common feature of many regulatory cells is their ability to produce IL-10, a cytokine that may create a microenvironment that facilitates regulation and may function to enhance the generation and function of regulatory immune cells.[82]

Every immunosuppressive regimen affects the balance between tolerance and rejection. Induction therapy with depleting antibodies generates a prolonged leucopenia, which is followed by repopulation. This has the potential to tip the balance in favour of immune regulation. Both ATG and alemtuzumab have been shown to induce regulatory T cells.[83,84] Basiliximab, a monoclonal antibody specific for CD25, on the other hand, might have a less beneficial effect on regulatory T cells, as these cells express high levels of CD25. In maintenance immunosuppression, mTOR inhibitors are of particular interest, because rapamycin has been shown to promote expansion of regulatory T cells.[85]

New therapies aiming to promote tolerance can be divided into two groups: those in which regulatory cells are directly infused and those in which regulatory immune cell production is induced. Cellular therapies, in which regulatory immune cells are administered to patients directly, are being studied in the context of graft-versus-host disease (GVHD). Preliminary results in humans show a slight reduction in the incidence of GVHD, without loss of the graft-versus-leukaemia effect or significant safety concerns.[86] A multicentre phase I/II study[87] will investigate the safety of infusing regulatory T cells into renal transplant recipients. The alternative approach, stimulating regulatory immune cell production in humans, was effective in mice suffering from GVHD who were administered IL-2 and rapamycin. Low-dose IL-2 therapy was also used successfully to treat patients with chronic GVHD.[88] It remains to be determined whether the same holds true for recipients of solid organ transplantation.

CONCLUSION

In the past century, enormous steps have been taken in the field of solid organ transplantation. Heart, lung and liver transplantations are life-saving. In renal transplantation, the life expectancy of transplant patients easily exceeds that of dialysis patients. All this has been made possible because of a drastic expansion in the immunosuppressive repertoire. Unfortunately, the side effects of these drugs can be severe, which is one of the reasons that life expectancy of renal transplant patients still falls significantly short of that of the general population. Expanding our understanding of the human immune system will hopefully provide us with newer, smarter drugs that promote immunotolerance without the side effects observed today.

REFERENCES

1. Morris PJ. Transplantation – a medical miracle of the 20th century. N Engl J Med. 2004;351:2678-80.
2. Sayegh, MH, Carpenter CB. Transplantation 50 Years Later – Progress, Challenges, and Promises. N Engl J Med. 2004;351:2761-6.
3. Schwartz R, Eisner A, Dameshek W. The effect of 6-mercaptopurine on primary and secondary immune responses. J Clin Invest. 1959;38:1394-403.
4. Schwartz R, Dameshek W. Drug-induced immunological tolerance. Nature. 1959;183:1682-3.
5. Murray JE, Merrill JP, Harrison JH, Wilson RE, Dammin GJ. Prolonged survival of human-kidney homografts by immunosuppressive drug therapy. N Engl J Med. 1963;268:1315-23.
6. Tilney NL. Transplant: from myth to reality. New Haven, Conn.: Yale University Press, 2003.
7. Hamilton D. Kidney transplantation: a history. In: Morris PJ, ed. Kidney transplantation: principles and practice. 5th ed. Philadelphia: W.B. Saunders, 2001:1-8.
8. Hamilton D. Reaching for the impossible: the quest for tissue replacement. In: Ginns LG, Cosimi AB, Morris PJ, eds. Transplantation. Boston: Blackwell Science, 1999:1-19.
9. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med. 2005;353:1711-23.
10. Hafezi-Moghadam A, Simoncini T, Yang Z, et al. Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. Nat Med. 2002;8:473-9.
11. Fauci AS, Dale DC, Balow JE. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. Ann Intern Med. 1976;84:304-15.
12. ten Berge RJ, Sauerwein HP, Yong SL, Schellekens PT. Administration of prednisolone in vivo affects the ratio of OKT4/OKT8 and the LDH-isoenzyme pattern of human T lymphocytes. Clin Immunol Immunopathol. 1984;30:91-103.
13. Cohen JJ, Duke RC. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. J Immunol. 1984;132:38-42.

14. Lanza L, Scudeletti M, Puppo F, et al. Prednisone increases apoptosis in in vitro activated human peripheral blood T lymphocytes. Clin Exp Immunol. 1996;103:482-90.

15. Cupps TR, Gerrard TL, Falkoff RJ, Whalen G, Fauci AS. Effects of in vitro corticosteroids on B cell activation, proliferation, and differentiation. J Clin Invest. 1985;75:754-61.

16. Pascual J, Royuela A, Galeano C, Crespo M, Zamora J. Very early steroid withdrawal or complete avoidance for kidney transplant recipients: a systematic review. Nephrol Dial Transplant. 2012;27:825-32.

17. Knight SR, Morris PJ. Steroid avoidance or withdrawal after renal transplantation increases the risk of acute rejection but decreases cardiovascular risk. A meta-analysis. Transplantation. 2010;89:1-14.

18. ten Berge RJ, Schellekens PT, Surachno S, The TH, ten Veen JH, Wilmink JM. The influence of therapy with azathioprine and prednisone on the immune system of kidney transplant recipients. Clin Immunol Immunopathol. 1981;21:20-32.

19. ten Berge RJ, Schellekens PT, Surachno S, The TH, ten Veen JH, Wilmink JM. A longitudinal study on the effects of azathioprine and high doses of prednisone on the immune system of kidney-transplant recipients. Clin Immunol Immunopathol. 1982;24:33-46.

20. Tiede I, Fritz G, Strand S, et al. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. J Clin Invest. 2003;111:1133-45.

21. Janeway CA. Immunobiology. New York, NY: Garland Publishing, 2001.

22. Halloran PF. Immunosuppressive drugs for kidney transplantation. N Engl J Med. 2004;351:2715-29.

23. Knechtle SJ, Kwun J, Iwakoshi N. Prevention trumps treatment of antibody-mediated transplant rejection. J Clin Invest. 2010;120:1036-9.

24. Liu EH, Siegel RM, Harlan DM, O'Shea JJ. T cell-directed therapies: lessons learned and future prospects. Nat Immunol. 2007;8:25-30.

25. Vincenti F, Kirk AD. What's next in the pipeline. Am J Transplant. 2008;8:1972-81.

26. Guba M, Graeb C, Jauch K, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. Transplantation. 2004;77:1777-82.

27. Randall T. New antirejection drugs anticipated. JAMA. 1990;264:1225.

28. Allison AC, Eugui EM, Sollinger HW. Mycophenolate mofetil (RS-61443): Mechanisms of action and effects in transplantation. Transplant Rev. 1993;7:129-40.

29. Eugui EM, Almquist SJ, Muller CD, Allison AC. Lymphocyte-selective cytostatic and immunosuppressive effects of mycophenolic acid in vitro: Role of deoxyguanosine nucleotide depletion. Scand J Immunol. 1991;33:161-73.

30. Platz KP, Sollinger HW, Hullett DA, Eckhoff DE, Eugui EM, Allison AC. RS-61443, a new, potent immunosuppressive agent. Transplantation. 1991;51:27-31.

31. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U.S. Renal Transplant Mycophenolate Mofetil Study Group. Transplantation. 1995;60:225-32.

32. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. Transplantation. 1996;61:1029-37.

33. Remuzzi G, Lesti M, Gotti E, et al Mycophenolate mofetil versus azathioprine for prevention of acute rejection in renal transplantation (MYSS): a randomised trial. Lancet. 2004;364:503-12.

34. Remuzzi G, Cravedi P, Costantini M, et al Mycophenolate mofetil versus azathioprine for prevention of chronic allograft dysfunction in renal transplantation: the MYSS follow-up randomized, controlled clinical trial. J Am Soc Nephrol. 2007;18:1973-85

35. Elion GB, Callahan S, Nathan H. Potentiation by inhibition of drug degradation: 6-substituted purines and xanthine oxidase, Biochem Pharmacol. 1963;12:85.

36. Marcén R. Immunosuppressive drugs in kidney transplantation: impact on patient survival, and incidence of cardiovascular disease, malignancy and infection. Drugs. 2009;69:2227-43.

37. Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature. 1992;357:695-7.

38. Kopp JB, Klotman PE. Cellular and molecular mechanisms of cyclosporin nephrotoxicity. J Am Soc Nephrol. 1990;1:162-79.

39. Ekberg H, Tedesco-Silva H, Demirbas A, et al ELITE-Symphony Study: Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med. 2007;357:2562-75.

40. Webster A, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus cyclosporin as primary immunosuppression for kidney transplant recipients. Cochrane Database Syst Rev. 2005;Oct 19;4:CD003961.

41. Nourse J, Firpo E, Flanagan W, et al. Interleukin-2-mediated elimination of the p27Kip1 cyclin-dependent kinase inhibitor prevented by rapamycin. Nature. 1994;372:570-3.

42. Andoh TF, Lindsley J, Franceschini N, Bennett WM. Synergistic effects of cyclosporine and rapamycin in a chronic nephrotoxicity model. Transplantation. 1996;62:311-6.

43. Gallon L, Perico N, Dimitrov BD, et al. Long-term renal allograft function on a tacrolimus-based, pred-free maintenance immunosuppression comparing sirolimus vs. MMF. Am J Transplant. 2006;6:1617-23.

44. Nashan B, Gaston R, Emery V, et al. Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients. Transplantation. 2012;93:1075-85.

45. Havenith SH, Yong SL, van Donselaar-van der Pant KA, van Lier RA, Ten Berge IJ, Bemelman FJ. Everolimus-Treated Renal Transplant Recipients Have a More Robust CMV-Specific CD8+ T-Cell Response Compared With Cyclosporine- or Mycophenolate-Treated Patients. Transplantation. 201315;95:184-91.

46. Webster AC, Lee VW, Chapman JR, Craig JC. Target of rapamycin inhibitors (sirolimus and everolimus) for primary immunosuppression of kidney transplant recipients: a systematic review and meta-analysis of randomized trials. Transplantation. 2006;81:1234-48.

47. Guerra G, Ciancio G, Gaynor JJ. Randomized trial of immunosuppressive regimens in renal transplantation. J Am Soc Nephrol. 2011;22:1758-68.

48. Lebranchu Y, Thierry A, Thervet E, et al, Efficacy and Safety of Early Cyclosporine Conversion to Sirolimus with Continued MMF—Four-Year Results of the Postconcept Study. Am J Transplant. 2011;11:1665-75.

49. Schena FP, Pascoe MD, Alberu J, et al. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. Transplantation. 2009;87:233-42.

50. Budde K, Becker T, Arns W, et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. Lancet. 2011;377:837-47.

51. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. Leukemia. 2007;21:1387-94.

52. Brennan DC,Flavink K, Lowell JA, et al. A randomized, double-blinded comparison of Thymoglobulin versus Atgam for induction immunosuppressive therapy in adult renal transplant recipients. Transplantation. 1999;67:1011-8.

53. Havenith SH, Remmerswaal EB, Bemelman FJ, et al. Rapid T cell repopulation after rabbit anti-thymocyte globulin (rATG) treatment is driven mainly by cytomegalovirus. Clin Exp Immunol. 2012;169:292-301.

54. Hale G. The CD52 antigen and development of the CAMPATH antibodies. Cytotherapy. 2001;3:137-43.

55. Bloom DD, Hu HZ, Fechner JH, Knechtle SJ. T-lymphocyte alloresponses of campath-1H-treated kidney transplant patients. Transplantation. 2006;81:81-7.

56. Cossburn M, Pace AA, Jones J, et al Autoimmune disease after alemtuzumab treatment for multiple sclerosis in a multicenter cohort. Neurology. 2011;77:573-9.

57. Thomas PG, Ishihara K, Vaidya S, Gugliuzza KK. Campath and renal transplant rejection. Clin Transplant. 2004;18:759-61.

58. Csapo Z, Benavides-Viveros C, Podder H, Pollard V, Kahan BD. Campath-1H as rescue therapy for the treatment of acute rejection in kidney transplant patients. Transplant Proc. 2005;37:2032-6.

59. Basu A, Remkumar M, Tan HP, et al. Reversal of acute cellular rejection after renal transplantation with Campath-1H. Transplant Proc. 2005;37:923-6.

60. Clatworthy MR, Friend PJ, Calne RY, et al. Alemtuzumab (CAMPATH-1H) for the treatment of acute rejection in kidney transplant recipients: long-term follow-up. Transplantation. 2009;87:1092-5.

61. van den Hoogen MW, Hesselink DA, van Son WJ, Weimar W, Hilbrands LB. Treatment of steroid-resistant acute renal allograft rejection with alemtuzumab. Am J Transplant. 2013;13:192-6.

62. Roberts DM, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. Transplantation. 2012;94:775-83.

63. Vo AA, Peng A, Toyoda M, et al. Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. Transplantation. 2010;89:1095-102.

64. Fuchinoue S, Ishii Y, Sawada T, et al, The 5-Year Outcome of ABO-Incompatible Kidney Transplantation With Rituximab Induction. Transplantation. 2011;91:853-7.

65. Jagadeesh D, Woda BA, Draper J, Evens AM. Post transplant lymphoproliferative disorders: risk, classification, and therapeutic recommendations. Curr Treat Options Oncol. 2012;13:122-36.

66. Tydén G, Genberg H, Tollemar J, et al. A randomized, double blind, placebo-controlled study of single-dose rituximab as induction in renal transplantation. Transplantation. 2009;87:1325-9.

67. Tydén G, Ekberg H, Tufveson G, Mjörnstedt L. A randomized, double-blind, placebo-controlled study of single dose rituximab as induction in renal transplantation: a 3-year follow-up. Transplantation. 2012;94:e21-22.

68. Clatworthy MR, Watson CJE, Plotnek G, et al. B-cell depleting induction. N Engl J Med. 2009;360:2683-5.

69. Nashan B, Moore R, Amlot P, Schmidt AG, Abeywickrama K, Soulillou JP. Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. Lancet. 1997;350:1193-8. [Erratum, Lancet 1997;350:1484.]

70. Kahan BD, Rajagopalan PR, Hall M. Reduction of the occurrence of acute cellular rejection among renal allograft recipients treated with basiliximab, a chimeric anti-interleukin-2-receptor monoclonal antibody. Transplantation. 1999;67:276-84.

71. Webster AC, Ruster LP, McGee R, et al. Interleukin 2 receptor antagonists for kidney transplant recipients. Cochrane Database Syst Rev. 2010;20:CD003897.

72. Morgan RD, O'Callaghan JM, Knight SR, Morris PJ. Alemtuzumab Induction Therapy in Kidney Transplantation: A Systematic Review and Meta-analysis. Transplantation. 2012;93:1179-88.

73. Knight RJ, Kerman RH, Schoenberg L, et al. The selective use of basiliximab versus thymoglobulin in combination with sirolimus for cadaveric renal transplant recipients at low risk versus high risk for delayed graft function. Transplantation. 2004;78:904-10.

74. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D. Thymoglobulin Induction Study Group Rabbit antithymocyte globulin versus basiliximab in renal transplantation. N Engl J Med. 2006;355:1967-77.

75. Brennan DC, Schnitzler MA, Long-term results of rabbit antithymocyte globulin and basiliximab induction. N Engl J Med. 2008;359[16]:1736-8.

76. http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2012/08/WC500130945.pdf.

77. Arora S, Tangirala B, Osadchuk L, Sureshkumar KK. Belatacept : a new biological agent for maintenance immunosuppression in kidney transplantation. Expert Opin Biol Ther. 2012;12:965-79.

78. Waiser J, Budde K, Schutz M, et al Comparison between bortezomib and rituximab in the treatment of antibody-mediated renal allograft rejection. Nephrol Dial Transplant. 2012;27:1246-51.

79. Sadaka B, Alloway RR, Woodle ES. Clinical and investigational use of proteasome inhibitors for transplant rejection. Expert Opin Investig Drugs. 2011;20:1535-42.

80. Rother RP, Rollins SA, Mojcik CF, Brodsky RA, Bell L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. Nat Biotechnol. 2007;25:1256-64.

81. Stegall MD, Chedid MF, Cornell LD. The role of complement in antibody-mediated rejection in kidney transplantation. Nat Rev Nephrol. 2012;8:670-8.

82. Wood KJ, Bushell A, Hester J. Regulatory immune cells in transplantation. Nat Rev Immunol. 2012;12:417-30.

83. Bloom DD, Chang Z, Fechner JH, et al. CD4+ CD25+ FOXP3+ regulatory T cells increase de novo in kidney transplant patients after immunodepletion with Campath-1H. Am J Transplant. 2008;8:793-802.

84. Lopez M, Clarkson MR, Albin M, Sayegh MH, Najafian N. A novel mechanism of action for anti-thymocyte globulin: induction of CD4+CD25+Foxp3+ regulatory T cells. J Am Soc Nephrol. 2006;17:2844-53.

85. Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. J Immunol. 2006;177:8338-47.

86. Brunstein CG. Miller JS, Cao Q, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood 2011;117:1061-70.

87. http://www.onestudy.org/

88. Shin H-J, Baker J, Leveson-Gower DB, Smith AT, Sega EI, Negrin RS. Rapamycin and IL-2 reduce lethal acute graft-versus-host disease associated with increased expansion of donor type CD4+CD25+Foxp3+ regulatory T cells. Blood. 2011;118:2342-50.