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# Vascular effects of immunosuppression

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Transplantation is the accepted treatment of end-stage organ failure. The introduction of cyclosporine (CsA) in the early 1980s greatly improved the outcome of solid organ transplantation, with an increase in 3-year survival from almost 40% to 70%.[1] Substantial advances in the development of additional immunosuppressants have allowed transplant physicians to more specifically modulate the immune response according to the precise requirements of both the transplanted organ and the patient receiving the allograft.[2] Although immunosuppressive agents are sufficient to minimize allograft rejection and promote short-term survival after transplantation, a major limitation to longer-term survival is the development of allograft vasculopathy (AV).[3–7]

Central to the development of AV is endothelial damage and subsequent dysfunction. Endothelial dysfunction contributes to the development of intimal hyperplasia and progressive plaque buildup that leads to AV.[8,9] Endothelial dysfunction is attributable to numerous factors, including organ preservation solutions, ischemia and reperfusion injury, acute allograft rejection episodes, dyslipidemia, hypertension, diabetes and the use of immunosuppressive drugs.[8,10] These factors elicit endothelial activation by disrupting the homeostatic balance between endothelium-derived relaxing factors such as nitric oxide (NO) and activating factors such as endothelin (ET-1).[9–11] The activated endothelium increases vascular resistance, adhesiveness, thrombogenicity and the risk of atherogenesis.[12]

Many of the currently used immunosuppressants cause endothelial dysfunction after transplantation and may further accelerate the development of intimal hyperplasia and AV. The objective of this review is to provide insight into the vascular effects of the most commonly used immunosuppressants: CsA, tacrolimus (Tac), rapamycin (Rapa) and its synthetic derivative everolimus (Erl), corticosteroids, azathioprine (AZA) and mycophenolate mofetil (MMF). We also provide a comparative discussion of the drugs' benefits and risks in terms of side effects and offer preventative strategies that emphasize tailoring of the immunosuppressive regimen to individual patient needs with modification as physiologic changes dictate.

## Immunosuppressants

### *Calcineurin inhibitors: cyclosporine and tacrolimus*

Cyclosporine (Sandimmune, Neoral; Novartis) is a cyclic peptide derived from a fungal product of *Tolypocladium inflatum gams*.[13] Tacrolimus (Prograf; Astellas) is a macrolide lactone compound, derived from a fungal product of *Streptomyces tsukubaensis*.[14,15] Both CsA and Tac are immunosuppressants commonly used to reduce the incidence and severity of allograft rejection after transplantation. Whereas Tac shares many pharmacologic characteristics with its predecessor CsA, these 2 drugs have different side effect profiles. For example, Tac is associated with less hirsutism and gum hyperplasia and may induce less nephrotoxicity and hypertension than CsA, but it is also associated with an

increase in posttransplantation diabetes mellitus.[2,13,16]

Both CsA and Tac are calcineurin inhibitors (CNIs). The drugs enter the cell by diffusion but bind to different immunophilins: CsA to cyclophilin and Tac to FK506 binding protein (FKBP)-12. The resulting complex binds to calcineurin, a phosphatase that under normal conditions dephosphorylates molecules such as nuclear factor of activated T cells (NFAT). Dephosphorylated NFAT enters the nucleus, where it binds to sites in the promoter regions of several cytokine genes, including interleukin (IL)-2.[15,17] Thus, by blocking calcineurin, both CsA and Tac inhibit IL-2 transcription. A central advantage of CNIs lies in their selective actions on the immune system, without affecting other rapidly proliferating cells.[18,19]

### *Proliferation signal inhibitors: rapamycin and everolimus*

Rapamycin (Sirolimus, Rapamune; Wyeth) is a product of the soil actinomycete *Streptomyces hygroscopicus*. It is a macrolide lactone immunosuppressant, similar in structure to Tac.[14] Rapamycin binds to the same family of immunophilins as Tac, the FKBPs, but instead of blocking calcineurin-dependent T-cell activation, the FKBP–Rapa complex inhibits the mammalian target of Rapa (mTOR) kinase, which is responsible for the phosphorylation of proteins involved in cell-cycle regulation and thus plays a critical role in transmitting signals to stimulate lymphocyte proliferation.[13,17] By inhibiting mTOR, Rapa interrupts cell cycling, causing arrest between $G_1$ and S phases.[9] Thus, Rapa inhibits the response to IL-2 and blocks lymphocyte activation, whereas the CNIs inhibit the earlier step of IL-2 production.[17] In combination with CsA, Rapa has been shown to reduce acute cellular rejection and AV compared with CsA and AZA combinations.[20]

Owing to its effects on cell cycling, Rapa acts as an antiproliferative agent. Normally, activation of mTOR signals proliferation of both smooth muscle and endothelial cells. Thus, Rapa has been shown to prevent arterial smooth muscle and endothelial proliferation, graft atherosclerosis and intimal hyperplasia after vascular injury.[13,14,21] Everolimus (Certican, RAD; Novartis) is an analog of Rapa and has been shown by intravascular ultrasound to reduce acute rejection and AV.[13,20,22] Though Rapa and Erl have not been compared head to head, it is thought that their therapeutic benefit and side effect profiles are similar.

### *Antiproliferative agents: azathioprine and mycophenolate mofetil*

Azathioprine (Imuran; GlaxoSmithKline) is a prodrug that is converted rapidly to 6-mercaptopurine, which is further converted to its active metabolite, thioinosine monophosphate. Thioinosine monophosphate is converted into a purine analog and incorporated into DNA, thus inhibiting its synthesis and the proliferation of both T and B lymphocytes. Azathioprine is used as maintenance therapy in combination with steroids and a CNI. A major side effect of AZA is bone marrow suppression, including leukopenia, anemia and thrombocytopenia.[13]

Mycophenolate mofetil (CellCept; Roche) is a noncompetitive inhibitor of inosine monophosphate dehydrogenase, a key enzyme in the de novo synthesis pathway of guanine nucleotides for lymphocytes.[9,13,14] Proliferating lymphocytes are dependent on this path because it is the only available one for purine synthesis and, thus, DNA replication. Other cells use both de novo and salvage pathways for purine synthesis and, thus, MMF acts as a selective inhibitor of lymphocyte proliferation.[13] Guanine is also necessary for the glycosylation of agranulocyte glycoproteins and, therefore, MMF also suppresses adhesion molecule glycosylation.[14] Similarly to AZA, MMF is used as an adjunct to standard antirejection therapy in renal, hepatic and cardiac transplantation recipients. Despite the higher tolerability and beneficial effects of MMF, it has not replaced AZA entirely, mainly owing to its considerably higher cost.[13,14,23]

### *Corticosteroids*

Steroids were among the first immunosuppressive agents used in clinical transplantation and remain an important element of both induction and maintenance regimens.[13] Steroids diffuse freely across cell membranes and bind to cytoplasmic receptors. The glucocorticoid receptor–steroid complex then translocates to the nucleus, where it binds to regulatory elements on DNA to inhibit binding. These actions result in altered expression of genes involved in immune and inflammatory responses, including those for growth factors, cytokines and adhesion molecules.[24] Corticosteroids are nonspecific and, therefore, affect the number, distribution and function of all types of leukocytes, including B and T lymphocytes and endothelial cells.[13]

Although corticosteroids are a standard component of immunosuppressive therapy in many transplantation recipients, they are associated with a large number of long-term adverse side effects. Hypertension, cataracts, gastric ulcers, poor wound healing and myopathy are all associated with steroid therapy. Important metabolic implications include hyperlipidemia, renal insufficiency, diabetes mellitus, osteopenia and chronic adrenal suppression.[13] Importantly, the side effects of the CNIs may be aggravated by the concomitant use of corticosteroids, so minimization of dosage or discontinuation of steroids from the therapy regimen in selected patients may help to improve the situation.[2,25]

## VASCULAR SIDE EFFECTS OF IMMUNOSUPPRESSION

### *Allograft rejection and immunosuppression*

Both CNIs (CsA and Tac) can rescue allografts from

refractory rejection, and maintenance immunotherapy with CNIs is associated with excellent 1-year patient survival statistics. Over time, the use of Tac as a maintenance immunosuppressive therapy has increased and is now about equal to that of CsA at 1-year after transplant.[4–7,13]

Although Tac shares a similar mode of activity with CsA, some studies report that the incidence of acute cellular rejection is lower with Tac.[26,27] In a prospective trial, Groetzner and colleagues[26] examined graft vessel disease by measuring new-onset lumen narrowing in a major coronary artery compared with baseline angiography. The incidence of disease 2 years after transplantation was comparable between CsA and Tac groups; however, the incidence of acute rejection and the overall number of rejection episodes was significantly higher among patients who received CsA. A recent multicentre trial confirmed this, finding an increase in both acute and recurrent rejection episodes with CsA versus Tac use,[27] which may have long-term effects given the association between rejection, AV and mortality.

Hollenberg and colleagues[28,29] proposed that endothelial impairment and dysfunction over time are predictive of later rejection and AV development. Several investigators have demonstrated that CNI exposure leads to endothelial dysfunction.[9,11,30–33] Clinical studies have shown that CsA treatment results in endothelial dysfunction in transplantation patients, finding impairment of forearm blood flow in patients give CsA compared with controls.[30–32] Data from our laboratory using a rodent model of immunosuppression revealed that CsA treatment impaired endothelium-dependent vasorelaxation of thoracic aortic rings.[11] Jeanmart and colleagues[9] had parallel findings with both their CsA and Tac experiments in an ex vivo porcine arterial model. Many other studies cited in both the basic and clinical scientific literature reported that Tac treatment preserved endothelial function[34–36] and prevented the development of intimal hyperplasia[37,38] relative to CsA.

Rapa and, on a larger scale, Erl have both been shown to reduce the incidence and severity of acute rejection and prevent AV in transplantation recipients.[20,22,39] The clinical literature is supported by animal models of Rapa immunosuppression in which this drug has demonstrated the ability to reduce and potentially reverse the development of AV.[40–42] Our laboratory data showed that, in contrast to CsA, Rapa treatment did not impair endothelial-dependent vasorelaxation, nor did it increase sensitivity to vasospasm.[43] This is an important finding because neither CsA nor Tac has been conclusively shown to prevent AV development,[33,44,45] which remains the limiting factor for long-term survival after transplantation.

### *Hypertension, dyslipidemia and immunosuppression*

The follow-up data from the multicentre trial by Grimm and colleagues[27] and the 5-year single-centre study by Kobashigawa and colleagues[46] showed that significantly fewer patients who received Tac than patients who received CsA experienced new-onset hypertension. In addition, the number of antihypertensive medications used to control blood pressure was significantly higher among patients who received CsA than among those who received Tac. These findings are fairly consistent in the clinical literature, with most centres noting decreases in effective blood pressure control and subsequent increases in antihypertensive treatments in patients on CsA-based immunosuppressive regimens.[13,26,27,47–49]

Cyclosporine-induced hypertension may develop from a variety of sources, including vascular dysfunction owing to direct cytotoxic effects on the endothelium, direct contractile effects on vascular smooth muscle cells, impaired NO release and/or increased ET-1 production.[9,11,50] Data from our laboratory implicate CsA-mediated endothelial dysfunction resulting from impaired NO and ET-1 homeostasis. A reduction in endothelial NO synthase (eNOS) protein expression is responsible for decreased NO production and further loss of a protective NO vasodilatory effect; increased sensitivity to ET-1 yields additional vasoconstriction.[11] One explanation for the lower rates of hypertension with Tac use is that, in contrast to CsA, Tac does not induce significant ET-1 production by the endothelium.[9,26,35] Petrakopoulou and colleagues[35] demonstrated that plasma ET-1 concentrations increased over time in patients taking CsA, whereas ET-1 levels significantly decreased over time in those taking Tac.

Although both CNIs are associated with dyslipidemic side effects, many reports agree that patients given Tac after transplantation have significantly lower serum cholesterol and triglyceride concentrations compared with those given CsA, thus requiring fewer lipid-lowering agents such as statins.[26,27,47,49] For example, Grimm and colleagues'[27] trial, as well as an earlier study by Taylor and colleagues,[49] showed that significantly fewer patients who received Tac required treatment for hyperlipidemia compared with those who received CsA. Although the widespread use of statins (for their beneficial effects on graft vascular function in addition to lipid profiles) now often brings the cholesterol issue to parity between the CNIs, there remains a general consensus that Tac has a safer profile than CsA with regard to maintenance of satisfactory lipid profiles.

Dyslipidemia is a serious side effect associated with proliferation signal inhibitors (PSIs). On its own, Rapa is associated with significant increases in both triglyceride and cholesterol levels.[51,52] Indeed, it may also exacerbate CsA-induced hyperlipidemia and hypercholesterolemia, steroid-induced hypertriglyceridemia and lipid disorders associated with renal disease when used in combination.[53] In addition, Erl potentiates the lipid disorders associated with CNI use.[22,54,55] These compounded effects synergistically increase endothelial dysfunction and intimal thickening, although

paradoxically lower rates of AV are noted with PSI use.

### *Renal dysfunction and immunosuppression*

Nephrotoxicity stems from renal vasoconstriction, another product of impaired vascular homeostasis seen with CNI use. Although CNI nephrotoxicity is well established and its incidence is similar with CsA and Tac, numerous studies have found it slightly lower with Tac use.

Groetzner and colleagues[26] found similar creatinine levels between groups, but they found that patients taking CsA needed more maintenance drugs. The trial by Grimm and colleagues[27] agrees that there is not much difference, but notes that the incidence of kidney disorders over time is slightly lower with Tac than CsA. Kobashigawa and colleagues'[46] 5-year results comparing Tac to CsA microemulsion found significant decreases in creatinine levels of patients taking Tac compared with those taking CsA, although the authors noted that the number of patients requiring hemodialysis over the 5-year period was similar between groups. They also noted that a decrease in target Tac trough blood levels may have accounted for this improvement in renal function in these patients.

Rapamycin and Erl are immunosuppressants with no direct impact on renal function; this is their most important advantage over CNIs. Recent studies have shown that switching from CNI- to PSI-based immunosuppression improves renal function in transplantation patients with CNI-related nephrotoxicity.[56,57] However, although Rapa and Erl do not directly impact renal function, they can potentiate CNI-induced nephrotoxicity, requiring dose reduction in CNIs to preserve renal function.[20,22,51,58,59]

### *Glucose tolerance and immunosuppression*

Glucose intolerance after transplantation occurs more frequently with Tac than with CsA.[2] In addition, Tac is associated with an increased risk of new-onset type 1 diabetes mellitus.[2,60] Diabetes mellitus contributes to peripheral and cerebral vascular disease, renal disease and complications with therapy and adherence. In addition, it further accelerates endothelial dysfunction due to accumulation of waste products and reactive oxygen species,[26,60] which further uncouples eNOS and elicits a further decrease in NO production. Grimm and colleagues'[27] multicentre trial results agree, demonstrating that although fasting glucose levels were elevated comparably in both treatment groups, double the number of patients taking Tac required insulin therapy than those taking CsA. Many centres note that high steroid dose remains the most important risk factor for glucose metabolism disorders and that patients taking Tac-based therapies can be successfully weaned from corticosteroid treatment to help improve this problem.[61–63]

Although most reports indicate that PSIs have no effect on glucose metabolism,[64,65] a recent study found that long-term Rapa treatment reduced insulin sensitivity and increased peripheral insulin resistance.[66] The lack of previous evidence may be related to the definition of posttransplantation diabetes mellitus by insulin requirements and not by the use of a glucose tolerance test, which is a better indicator of glycemic abnormalities, as in the study by Teutonico and colleagues.[66]

## PREVENTION OF MORBIDITY

The metabolic abnormalities discussed mainly result from the immunosuppression necessary to prevent allograft rejection. Just as importantly, however, these disturbances can themselves contribute further to the development of AV. Therefore, tailoring the therapeutic regimen to suit an individual patient's history and needs is required to minimize comorbidity and protect from further endothelial damage and AV progression. Preventative therapy must be initiated early, particularly since most of the intimal thickening that leads to AV occurs during the first year after transplantation.[2]

### *Induction therapy and delayed CNI administration*

A common strategy of immunosuppression is induction therapy, which employs initial lymphocyte depletion or IL-2 receptor antagonism with agents such as monoclonal or polyclonal antibodies.[13] This strategy provides effective protection against rejection in the first critical weeks after transplantation. Corticosteroids are regularly given in conjunction, but their long-term use should be minimized owing to side effects such as diabetes mellitus and hypertension,[13,25,60] both of which perpetuate a vicious circle of endothelial dysfunction and intimal thickening that lead to AV. Maintenance immunosuppression usually includes a CNI and an adjunctive agent such as AZA or MMF. A study by Cantarovich and colleagues[67] described the use of antithymocyte globulin induction therapy to permit a delay in CsA initiation in heart transplantation patients with renal dysfunction, without compromising the efficacy of immunosuppression.

### *Switching CNIs*

Either CsA or Tac can be used safely and effectively as maintenance immunotherapy for transplantation recipients. However, many centres prefer Tac, particularly for patients with a high risk of rejection such as those with ABO-incompatibility, delayed graft function, sensitization and other risk factors such as hypertension and dyslipidemia.[2,13,16,25] Conversion from CsA to Tac is also frequently used to treat recurrent rejection episodes. Cyclosporine is often preferred for patients who experience Tac-related adverse events such as diabetes mellitus, chest pain, tremor, gastrointestinal symptoms or encephalopathy.[13,68]

### *Balancing PSIs and CNIs*

Rapamycin, in combination with CsA, effectively reduces the incidence of acute allograft rejection.[51,58] Owing to the synergistic effect of PSIs on CNI-induced nephrotoxicity, prolonged combination of the 2 drugs without dose reduction of the CNI may lead to progressive renal damage.[20,22,58,59] Using low-dose CNI regimens with PSIs, early elimination of CNI therapy or complete CNI avoidance may be a reasonable strategy in select patients. Ultimately, the development of newer immunosuppressive agents is allowing for greater tailoring of therapy to each individual patient's immunologic risk and side effect profile.

### *Statins and angiotensin-converting enzyme inhibitors*

The beneficial impact of drugs such as statins or angiotensin-converting enzyme (ACE) inhibitors on endothelial function in coronary patients is well established. A pilot study in 1995 by Kobashigawa and colleagues[69] found that after heart transplantation, pravastatin had beneficial effects on cholesterol levels, the incidence of rejection, 1-year survival and the incidence of AV. Moreover, the benefit of statins has been underscored by recent observations that their administration preserves endothelial function and inhibits neovascularization. The 10-year follow-up to the Kobashigawa study suggests that continued use of statins in transplantation patients maintains a survival benefit and appears to reduce longer-term development of AV.[70] In addition to their endothelial benefits, statins can reduce the hyperlipidemia associated with CNI and PSI use.

## CONCLUSION

An optimal immunosuppressive regimen must be selective and specific, with the combination acting synergistically to allow the recipient's immune system to tolerate the graft. The current roster of immunosuppressive agents only partially meets these criteria. The CNIs act primarily on T lymphocytes, thus making them better in terms of selectivity than other drug families such as the antiproliferatives. Unfortunately, CNIs are associated with many adverse side effects. The second generation of selective agents, the PSIs, are more active toward signal transduction in lymphocytes but still inhibit responses to a variety of cytokines, thus compromising selectivity. In addition, although PSIs seem superior in terms of preventing comorbidities that have long been associated with CNI use, caution must be exercised in dosing during CNI coadministration so that the synergism of the drugs does not exacerbate CNI-related side effects such as nephrotoxicity.

Ultimately, clinicians must decide the best means of optimizing therapy for individual patients based on risk factors such as rejection, delayed graft function, ABO-incompatibility and other adverse events such as diabetes mellitus, hypertension, dyslipidemia and cosmetic changes. By assessing the risks for each individual patient, the physician can choose the most appropriate immunosuppressive "cocktail" initially and adjust the regimen accordingly to provide optimal immunosuppression.

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# The MacLean–Mueller Prize

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Each year the *Canadian Journal of Surgery* offers a prize of $1000 for the best manuscript written by a Canadian resident or fellow from a specialty program who has not completed training or assumed a faculty position. The prize-winning manuscript for the calendar year will be published in an early issue the following year, and other submissions deemed suitable for publication may appear in a subsequent issue of the Journal.

The resident should be the principal author of the manuscript, which should not have been submitted or published elsewhere. It should be submitted to the *Canadian Journal of Surgery* no later than Oct. 1.

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Le *Journal canadien de chirurgie* offre chaque année un prix de 1000 $ pour le meilleur manuscrit rédigé par un résident ou un fellow canadien d'un programme de spécialité qui n'a pas terminé sa formation ou n'a pas accepté de poste d'enseignant. Le manuscrit primé au cours d'une année civile sera publié dans un des premiers numéros de l'année suivante et les autres manuscrits jugés publiables pourront paraître dans un numéro ultérieur du Journal.

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