Chapter 10
Allograft Survival with Calcineurin Inhibitors

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10.1 Introduction

The immunosuppressive drugs cyclosporine A (CsA) and tacrolimus (FK506), also called calcineurin inhibitors, have truly revolutionized allograft transplantation. The introduction of CsA in 1976 was the first major advance in transplantation since the introduction of prednisone and azathioprine made allograft transplantation possible in the early 1950s and 1960s. FK506 was approved in 1994 and led to dramatic improvements in solid organ transplantation, allowing highly antigenic lymph node bearing allografts, such as the small bowel, to be transplanted. Recently, FK506 monotherapy has successfully allowed combined small bowel and partial abdominal wall transplantation in humans. The success of FK506 and CsA has made them key drugs in the modern era of transplantation. The purine synthesis inhibitor mycophenolate mofetil (MMF) was approved in 1995, and the drug Sirolimus (rapamycin) was introduced in 1999. Combining these drugs with calcineurin inhibitors has significantly reduced the incidence of acute rejection and improved solid organ allograft survival, with a reduction in adverse effects.

10.2 Molecular Action of Calcineurin Inhibitors

Cyclosporine A is a neutral lipophilic cyclic undecapeptide isolated from the fungus *Tolypocladium inflatum*, whereas FK506, also known as tacrolimus, is a macrolide lactone produced from the fermentation broth of *Streptomyces tsukubaensis*. Calcineurin inhibitors exert their effects by regulation of cytokine production. Cytokine production in T cells is regulated by a series of steps involving phosphorylation and dephosphorylation of specific proteins. Both CsA and FK506 are prodrugs, because they must first form a complex with cellular proteins called “immunophilins” before exerting their effects. There are two classes of immunophilins, the FK506 binding proteins (FKBP) which bind tacrolimus and sirolimus, and the cyclophilins (CyP) which bind CsA. Calcineurin is a phosphatase that is crucial for intracellular events leading to interleukin-2 (IL-2) gene transcription and release.
by T cells. Once the drugs bind to their respective immunophilin, the drug-immunophilin complex inhibits the activity of calcineurin, thereby reducing the production of IL-2 and other cytokines. Both CsA and FK506 block T-cell proliferation by mechanisms that involve the inhibition of the key signaling phosphatase calcineurin, hence their name calcineurin inhibitors. Inhibition of calcineurin activation by CsA and FK506 blocks T-cell receptor (TCR)-mediated production of interleukin-2 (IL-2), a growth factor critical for T-cell proliferation. Blocking T-cell proliferation prevents the immune system from effectively reacting against alloantigens. Recent studies suggest that the effects of these drugs are not limited to blocking calcineurin activation and IL-2 production. Both CsA and FK506 have been shown to modulate the production of various cytokines and growth factors, including transforming growth factor-β (TGF-β), interferon γ, TNF-α, IL-2, IL-4, IL-5, and IL-13. By potentiating the expression of the potent immunosuppressive cytokine TGF-β, TGF-β has been implicated in mediating at least some of the effects and toxicities of CsA and FK506. Another calcineurin-independent mechanism has been shown to be the blockade of epidermal growth factor receptor induced cell growth.

FK506 has been shown to be 100 times more potent than CsA. There are four intracellular binding proteins (immunophilins) for FK506 – FKBP−12, −13, −25, and −52, the first being the most important protein crucial for T-cell activation. Binding of FK506 to FKBP-12 receptor protein prevents activation of T cells and is also a potent suppressor of B-cell activation.

Both FK506 and CsA have similar renal and hepatic toxicities but differ in their other toxic side effects, presumably because of differences in the biological actions of their binding proteins. Neurotoxicity and diabetes can complicate the use of FK506. CsA can cause hypercholesterolemia, increasing the risk of cardiovascular complications in recipients receiving maintenance immunosuppression. FK506 possesses other positive effects and has been shown to promote nerve regeneration in small animal and large animal models and in humans after nerve injury.

### 10.2.1 Immunosuppression with Calcineurin Inhibitors and Survival of Composite Tissue Allografts in Small Animals

Hewitt et al. were the first to show that rejection could be prolonged in a rat hindlimb transplantation model by using a short-term course of CsA 25 mg/kg for 20 days with one animal probably developing tolerance showing no signs of rejection for 701 days after transplantation. Long-term intermittent immunosuppression with CsA 8 mg/kg twice weekly produced long-term survival for more than 400 days in three animals, and long-term continuous immunosuppression with CsA 8 mg/kg/day produced four animals in which rejection could be prevented for greater than 200 days. Interestingly, 19.7% of the lymphocytes in the peripheral blood and spleen of these long-term survivors were donor derived, indicating that