Transpl Int (1996) 9: 527–534

EDITORIAL

Barry D. Kahan

The three fates of immunosuppression in the next millenium: selectivity, synergy, and specificity

Received: 4 July 1996
Accepted: 8 July 1996

B. D. Kahan
Division of Immunology and
Organ Transplantation,
Department of Surgery,
The University of Texas Medical School
at Houston, 6431 Fannin, Suite 6.240,
Houston, TX 77030, USA
Fax: + 1 713 792 5917

Introduction

In classical mythology, the three Fates, who are portrayed in art and poetry as weavers, worked in concert to assign to each human his destiny. Clotho spun the thread of life, Lachesis decided its span, and Atropos cut the thread at death. Not one of these three goddesses, by her individual action, could complete the task alone. Likewise, at the dawn of the 21st century, the archetypal immunosuppressive regimen will most certainly exploit a triumvirate of effects – selectivity, synergy, and specificity – which, together with immunological maneuvers, will induce a state of immunological tolerance (or "partial" tolerance) and, thus, ensure the future success of human solid organ transplants.

Early in this century it was established that host immune responses toward foreign donor antigens trigger allograft rejection [24, 25, 37, 46]. Interest in clinical immunosuppression has grown rapidly since midcentury, when Hamburger et al. [19] applied total body irradiation to facilitate the acceptance of human renal transplants. In the 1960s, based upon the work of Schwartz and Dameshek [50], Calne et al. [6] used the first "designer drug", 6-mercaptopurine [23], and its more consistently bioavailable nitroimidazole derivative, azathioprine (Imuran, Burroughs-Wellcome, USA) [41], to inhibit multiple enzymes in both the de novo and the salvage pathways of purine synthesis.

The era of chemical immunosuppression began to flourish in the 1970s, when azathioprine was coadministered with glucocorticoids, which had been shown to cause lymphocytopenia, thymic atrophy, and anti-inflammatory effects, as well as to reduce the incidence and reverse the progression of allograft rejection episode. However, this immunosuppressive regimen inhibited nonselective elements of host resistance, such as monocytes, granulocytes, and macrophages. Because this profound depression of host resistance led to an excessive incidence of serious infections, particularly when combined with polyclonal antilymphocyte sera, there was an urgent need for immunosuppressants that would inhibit T (and/or B) cells selectively.

The 1980s witnessed the development of two pivotal T-cell-selective agents that acted without producing perceptible effects on nonspecific host resistance: the anti-CD3 monoclonal antibody (mAb) OKT3 [15] became the most effective agent to reverse acute allograft rejection episodes, and cyclosporin (CyA, Sandimmune, Sandoz Pharma, Switzerland) [7], a lymphokine synthesis inhibitor, quickly became the cornerstone of maintenance immunosuppression. In spite of its effectiveness, the utility of CyA, like that of its mechanistic analog tacrolimus (FK 506; Prograf, Fujisawa, Japan) [42], has been limited by the agent's pleiotropic array of adverse side effects.

Future advances in immunosuppression hinge on the development of synergistic regimens in which immunosuppressants selective for host T- and B-cell alloimmune responses are combined. The results of a recent clinical trial of such a regimen in humans showed that the combination of subtherapeutic doses of CyA and sirolimus (SRL, rapamycin; Rapamune, Wyeth-Ayerst, USA) [8] reduced the incidence of acute rejection episodes to less than 10 %, mitigated the toxic effects of large individual drug doses, and permitted early withdrawal of steroids

from the immunosuppressive regimen. However, the ultimate goal is to discover a therapeutic regimen that not only acts synergistically and selectively, but also specifically, to induce a state of immunological tolerance in which only the destructive antidonor responses are disrupted within the host's immune repertoire.

Selectivity

An ideal immunosuppressant would selectively disrupt the antigen presentation properties of dendritic and monocyte/macrophage cells (APCs) without affecting their chemotactic, phagocytic, and oxidative properties, which are important for the destruction of microbial pathogens. Although several xenogeneic mAbs bind to and inactivate surface coreceptors, they cannot be used for chronic treatment because they must be delivered intravenously and their administration induces the production of neutralizing host antibodies. Of greater promise are the receptor conjugates which are capable of selectively binding to designated surface receptor epitopes, resulting in target cell lysis or activation, particularly the ligand-immunoglobulin-conjugates as opposed to the ligand-diphtheria-toxin-linked reagents, the utility of which is limited by widespread host pre-immunization by diphtheria-pertusis-tetanus vaccination. An alternate approach to blocking pre-existent surface co-receptor moieties that mediate antigen presentation is the inhibition of their synthesis by the administration of antisense oligonucleotides.

Antisense oligonucleotides

Antisense oligonucleotides (Oligos) produce selective actions on nonspecific elements of host resistance by inhibiting the expression of cellular genes viar arrest of translation, inhibition of processing, or promotion of degradation of the targeted RNA. In phosphorothioate Oligos (PS-Oligos), a sulfur atom is substituted for one of the nonbridging oxygen atoms in the phosphate backbone in order to enhance the Oligo's resistance to hydrolysis by the 3′ exonuclease RNase H. On the basis of the Watson-Crick base pairing principle, PS-Oligos were designd to target intercellular adhesion molecule-1 (ICAM-1), an immunoglobulin-related cell surface marker on APCs that binds to lymphocyte function-associated antigen-1 (LFA-1) [45], thereby augmenting immune activation via the T-cellreceptor (TcR) but not via the interleukin-2 receptor (IL-2R) pathway (Fig. 1). ICAM-1 upregulation affects not only alloactivation processes, but also acute inflammatory responses to cytokines. Just as studies showed that anti-ICAM-1 mAbs interrupt rejection processes [21] and blunt ischemic injuries [30], anti-ICAM-1 PS-Oligos demonstrate these same characteristics in rat renal transplant models [55]. Our phase I clinical trials in stable renal transplant recipients document that patients experience only modest adverse side effects after intravenous administration of PS-Oligos; thus, phase II studies are underway to test the efficacy of these molecules.

T-cell-selective pharmacological agents: cyclosporin, tacrolimus, and sirolimus

Because T cells play a pivotal role in the activation of both cellular and humoral responses, a significant advance in immunosuppressive therapy has been the development of T-cell-selective pharmacological agents, which halt rejection processes by selectively inhibiting the maturation and differentiation of alloreactive T- and B-cell immune elements. Although the new antiproliferative agents such as mizorbine (Bredinin, Tojo Pharma, Japan) [33] and mycophenolate mofetil (RS-61443, Syntex, USA) [43] act noncompetitively to inhibit inosine monophosphate dehydrogenase isozyme 2 more than other enzymes that catalyze purine synthesis and salvage, their selectivity is limited because the upregulation of isozyme 2 in nonlymphoid cells may result in drug-induced myelodepression, thereby impairing the elements of nonspecific resistance.

Therefore, the prototype of T-cell-selective agents remains CyA, which inhibits antigen-activated lymphokine synthesis and cytotoxic T-cell generation. Interestingly, the actinomycete macrolide TRL inhibits lymphokine synthesis by a mechanism apparently similar to that of CyA even though it binds to a different set of intracellular proteins ("immunophilins") and is structurally unrelated to CyA. CyA binds to cyclophilin, and TRL binds to FK binding protein (FKBP12) to form CyA-cyclophilin and TRL-FKBP12 complexes. In addition, although immunophilins act as isomerases, regulating protein folding by altering the cis-/trans relationships of peptidyl-prolyl bonds, the drug-immunophilin complexes produce a distinct effect: inhibition of the phosphatase action of calcineurin (Cn). Drug-immunophilin complexes bind to an overlapping zone where the latch region of the regulatory B (CnB) subunit interacts with and regulates the phosphatase action of the enzymatic A (CnA) subunit [18, 38]. The interaction has been dissected using chemical cross-linking, photoaffinity labeling, random mutagenesis, and, recently, x ray crystallographic structural analysis. The contribution of the CnB subunit confers specificity since neither protein phosphatase 1 nor 2 A bears a regulatory subunit. This allosteric effect prevents the enzyme-mediated dephosphorylation that is necessary for nuclear penetration of a regulatory protein of activated T cells (NFAT), an event which is necessary for T cell activation [35]. NFAT represents a unique biochemical target character-

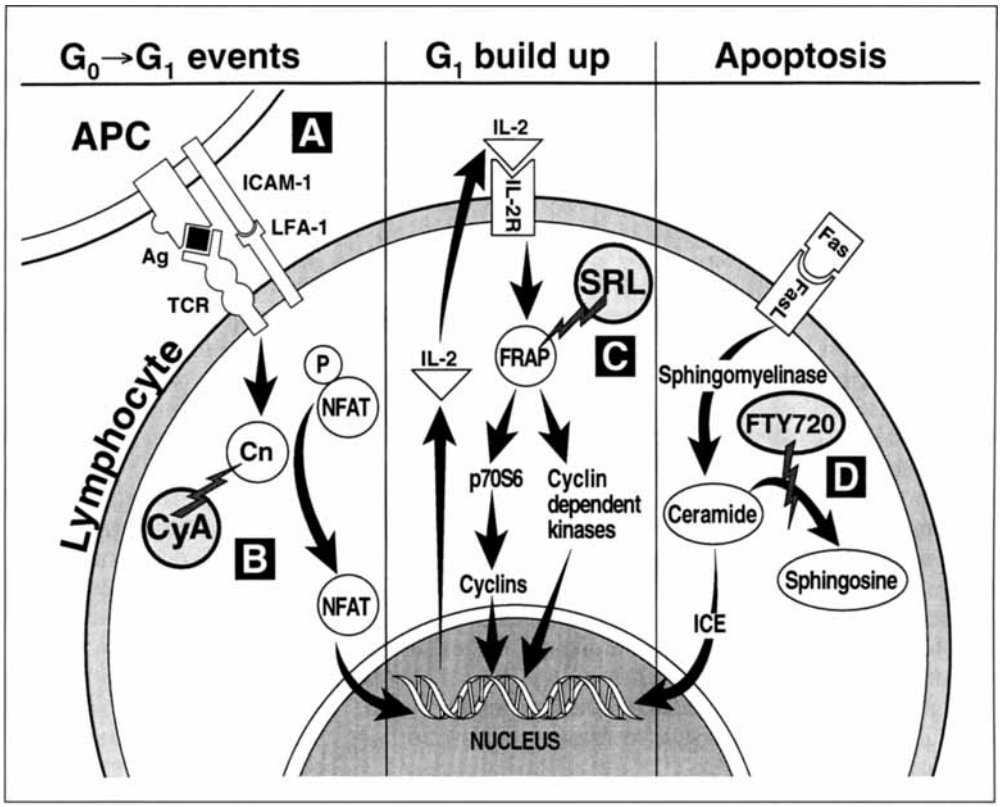

Fig. 1 Putative sites of T-cell-specific immunosuppressive agents. These agents can be classified based upon the phase of the cycle cell during which they act. In the G_0 to G_1 transition, intercellular adhesion molecule-1 *(ICAM-1)* antisense phosphorothioate oligonucleotide *(PS-Oligo)*, denoted by *A*, inhibits the antigen-presenting cell function of binding to lymphocyte function antigen-1 *(LFA-1)*, and cyclosporin *(CyA)* or tacrolimus *(TRL)* inhibits calcineurin *(Cn)*, denoted by *B*. During the G_1 build-up, sirolimus *(SRL)*, denoted by *C*, inhibits the FK-RAPA-associated protein *(FRAP)*. In the apoptotic pathway, FTY 720, denoted by *D*, may block ceramide degradation, thereby leading to ceramide accumulation

istic of T and possibly B cell [3] signaling pathways due to its restricted tissue distribution, in contradistinction to AP-1 and NF-κB, which are widely distributed among non-lymphoid cells. Further analysis of the molecular structures of immunophilin-immunosuppressant complexes that bind to the regulatory CnB sites is likely to provide insights into the design of new, more avid inhibitors.

However, two puzzling observations weaken the theory that Cn is the sole target of CsA and TRL immunosuppression. First, therapeutic concentrations of CsA only halve Cn activity measured with synthetic substrates [2]. Second, individual patients display distinct and very different ratios of therapeutic to toxic effects for CsA or for TRL. One might attempt to reconcile these apparently confounding observations with the Cn theory by hypothesizing that CsA and TRL display distinctive spectra of inhibition of the Cn isozymes α and β [16]. However Cn mutants are unable to discriminate inhibition by TRL-FKBP12 from that produced by CsA-CYP, suggesting that these two ligand-immunophilin complexes compete for the same binding site. Alternatively, the greater potency of TRL compared with CsA may relate to the role of its immunophilin FKBP to regulate calcium (Ca^{2+}) channels. FKBP anchors to the inositol-1,4,5-triphosphate receptors (IP3R) on endoplasmic reticulum, a critical pathway for signal transduction via the nonreceptor protein kinase fyn, following TcR-mediated phosphorylation. Thus, FKBP stabilizes dynamically regulated Ca^{2+} flux channels which are associated with ryanodine receptors and with IP3R in sarcoplasmic and endoplasmic reticulum [10]. Upon binding of TRL to FKBP12, disruption of calcium mobilization and/or homeostasis may potentiate Cn inhibition.

Indeed, the arrays of pleiotropic, particularly arteriolopathic and nephrotoxic, side effects of CyA and TRL prevent these agents from being administered in sufficient doses to exploit their full potentials in transplantation [26]. Because multiple analogs of CyA, including cyclosporin G and SDZ IMM-125, display similar toxic properties, the adverse side effect profile has not been dissociated from the immunosuppressive effects of CyA by molecular modifications. Serendipitously, it was discovered that SRL, a compound that is structurally but not mechanistically related to TRL, is a non-nephrotoxic T-cell-selective immunosuppressant. SRL inhibits cytokine signal transduction pathways by blocking an FKBP12-SRL-associated protein (FRAP), which is a phosphatidyl-inositol kinase that associates with several src-like and receptor-type kinases [47, 48]. The binding of SRL to FRAP inhibits activation of the kinase $p70^{s6k}$ (but not $p85^{s6k}$) [13], thereby preventing hyperphosphorylation of 40S ribosomal proteins [4, 9], an essential step in G_1 progression. In addition, SRL seems

to inhibit downstream cyclin-dependent kinases, including $p34^{cdc2}$ and $p33^{cdk2}$ [39], by promoting the translation of the negative regulator $p27^{kip1}$. Although the use of SRL monotherapy to prolong allograft rejection is currently being explored in European studies, we have been more intrigued by the immunosuppressive synergism produced by the combination of SRL with CyA.

Synergy

During the last decade, in a number of clinical settings, physicians have been prescribing synergistic combinations of drugs at individually subtherapeutic and nontoxic doses to avoid the considerable toxicity associated with the administration of potent drugs individually at relatively large doses. A synergistic drug combination was first described by Hitchings [22]: sulfamethoxazole and trimethoprin, two agents that act sequentially during microbial folate synthesis, exhibited markedly enhanced antibacterial potency. More recently, Kaplan and Hirsch [29] described a synergistic effect of combinations of agents that attack different stages of the human immunodeficiency virus (HIV) replication cycle, such as inhibitors of reverse transcriptase with inhibitors of virus-encoded protease.

Elucidation of the nature of drug interactions demands strict measurement of the dose-effect relationships for each drug alone and in combination. Chou and Talalay [12] used the mass action law to express the median effect equation and derive the combination index (CI), a parameter of drug interaction that has proven applicable in studies of immunosuppressive drugs [59], HIV [5], and cancer chemotherapy [44]. Interactions are considered additive if CI values equal 1.0, synergistic if they are less than 1.0, and antagonistic if they are greater than 1.0.

Our studies in rodents suggest that a combination of immunosuppressive agents that act during two sequential phases of the cell cycle, namely, the G_0 to G_1 transformation (CyA) and the G_1 build-up (SRL), displays a greater degree of immunosuppressive synergism than two agents that both act on the G_0 to G_1 transformation (CyA and anti-TcR MAb) [31], or two agents that interrupt the nonsequential G_0 to G_1 and S phases [CyA and brequinar (DuP785, DuPont-Merck, USA)] of the cell cycle [28].

The CyA/SRL Combination

Animal models: prophylaxis and treatment of rejection

The median effect analysis was used to show that SRL and CyA act synergistically to prolong the survival of rat heart and kidney transplants [53]. Furthermore, in rat recipients, the acute allograft rejection episodes that emerge after induction therapy with subtherapeutic doses of CyA (1.0 mg/kg per day) were reversed by treatment with SRL (0.08 mg/kg per day) beginning at the onset of the episode [60]. On the one hand, the results of quantitative competitive polymerase chain reactions document that SRL potentiates the inhibitory effects of CyA to reduce the levels of IFN-γ, IL-2, and IL-10 mRNA transcripts in rat heart allografts. On the other hand, because CyA serves to reduce the synthesis of lymphokines, lower SRL doses differentially inhibit lymphokine signal transduction [27].

Clinical trials: prophylaxis of rejection

Our phase IIA study showed that there was a modest (7%) incidence of acute rejection episodes in patients who received a concentration-controlled CyA/standard steroid dose regimen to which SRL was added. In addition, steroids could be withdrawn from the immunosuppressive regimens of these SRL-treated patients as early as 1 week after transplantation. Median effect analysis of the CyA/SRL combination documented that SRL acts synergistically with CyA in humans (CI = 0.1). In fact, the addition of SRL to subtherapeutic concentrations of CyA potentiated immunosuppressive activity to a level beyond that achieved with therapeutic amounts of CyA. A multicenter, randomized, placebo-controlled phase IIB trial also demonstrated that SRL treatment reduced the incidence of acute rejection episodes among all patients treated with full-dose CyA and among, nonblack patients treated with half-dose CyA. Moreover, our anecdotal report noted that the addition of SRL to a CyA-based regimen reversed ongoing acute cellular rejection in a human renal allograft recipient who was previously resistant to courses of equine antilymphocyte globulin and OKT3 [51].

Challenges to the design of CyA/SRL combinations

The first challenge to the design of a successful CyA/SRL combination – the partial overlap of the agents' toxicity spectra – was discovered during clinical trials. Although SRL is not nephrotoxic and does not potentiate the hypertensive effects of CyA, it shares its hyperlipidemic predilections. CyA primarily produces hypercholesterolemia, and SRL, hypertriglyceridemia [40]. Moreover, both agents are potentially hepatotoxic; whereas CyA tends to increase serum glutamic pyruvic transaminase levels, SRL increases serum glutamic oxalacetic transaminase values. These adverse effects may be controlled by the use of low SRL doses, because even 0.5 mg/m^2 doses do not seem to attenuate the degree of immunosuppressive synergy.

The second challenge is the pharmacokinetic interaction between SRL and CyA, both of which are metabolized by the cytochrome P450 (CYP) IIIA4 isozyme. In rodents and in humans, CyA and SRL display a significant, albeit dose-dependent, pharmacokinetic interaction. Using ANOVA models and forward chaining to stratify drug concentrations independently, we showed that in humans the interaction could be minimized at SRL trough concentrations below 10 ng/ml and CyA average concentrations, defined as the quotient of the area under the concentration-time curve divided by the dosing interval [34], below 450 ng/ml. These findings were confirmed in a rat model; simultaneous gavage of the two drugs produced a 2- to 11-fold dose-dependent increase in blood and tissue concentrations. This pharmacokinetic effect and the immunological effect appeared to contribute equally to produce the synergistic interaction [56].

A third, and more serious, challenge to the design of a robust immunosuppressive regimen is the limited ability of SRL to overcome CyA-resistant rejection in the more stringent animal models, such as the rat small bowel graft versus host (GVH), mouse heart, and dog renal allotransplant models. Although SRL prolonged the survival of orthotopic (BN x Lewis) F_1 small bowel transplants in Lewis (LEW; RT-1^l) recipients across the host-versus-graft disparity, it had only a modest effect and did not act synergistically (CI $\approx$ 0.96) with CyA against the GVH reaction [54]. In CyA-resistant C3H (H-2^k) recipients of Balb-c (H-2^d) vascularized heterotopic heart allografts, coadministration of SRL with CyA only produced a modest synergistic interaction (CI = 0.65) [58]. Finally, because species-specific toxicities limit the amount of SRL tolerated by dogs and primates, only a modest degree of synergistic immunosuppression may be achieved in these models. Thus, in more stringent transplant situations, other drugs may need to be added to, or substituted for, one of the agents in the CyA/SRL combination.

Specificity

As we approach the next millennium, the primary goal of basic science research in organ transplantation remains the induction of full or at least "partial" immunological tolerance, which may vitiate or at least reduce the need for debilitating chronic immunosuppression. To induce tolerance without disrupting the entire immune repertoire, the host's responses must be specifically altered only with regard to the foreign antigenic epitopes present on donor tissue.

Apoptosis

Apoptosis, or programmed cell death, is a physiological process that regulates the immune response by triggering a suicide mechanism in cells. Selective apoptosis of host immune cells that are specifically reactive to donor-type foreign alloantigens may produce immunological tolerance by clonal deletion. One promising agent capable of producing this effect may be the sphingosine analog FTY 720, which is derived from the fungus *Isari sinclarii* [1]. This immunosuppressant prolongs the survival of rat cardiac and canine renal allografts [57]. Although the agent produces lymphocyte emigration and sequestration, more significantly, its addition, concomitant with antigenic stimulation in vitro, seems to drive cells toward apoptosis, as evidenced by the characteristic ladder degradation of DNA upon agarose gel electrophoresis [57]. Presumably, FTY 720 inhibits the breakdown of ceramide, a second messenger that links some apoptotic pathways [20] to activation of IL-1 β-converting enzyme-like proteases. Two natural apoptotic stimuli increase ceramide concentrations by triggering sphingomyelinases, namely, signal transduction via the Fas (CD95) receptor activates the acidic form [14], and via tumor necrosis factor (TNF)-α/TNFR-1, the neutral form [32] of sphingomyelinases. Induction of apoptosis by FTY 720 represents a unique mechanism of immunosuppressive drug action; little evidence suggests that other drugs induce apoptosis. Furthermore, CyA acts synergistically with FTY 720, perhaps due to its capacity to inhibit growth factor production, possibly producing an effect akin to factor deprivation, which causes the continuously expressed oncogene c-myc to generate an apoptotic signal. Although there is no information about the toxicity of the drug in humans, FTY 720 is metabolized by lysosomal and membrane enzymes as well as possibly by CYP IVA, but not by CYP IIIA4, the enzyme that primarily biotransforms CyA and SRL. These findings suggest that FTY 720 proffers a new therapeutic method for deleting ("stripping") specific T-cell clones.

Antigen-induced immunodeviation

Whereas apoptosis causes the deletion of donor-reactive cells, exposure of the host to the foreign donor alloantigenic epitopes in a fashion different from the way they are presented during organ transplantation may alter (immunodeviate) the host response from destructive to tolerogenic. Hosts develop a destructive response after they are sensitized by direct presentation of alloantigens on in situ donor dendritic cells. Compared with direct presentation, indirect antigen presentation through host APC processing of subcellular donor alloantigens represents a more tolerogenic route [11]. Treatment of hosts with native (or altered) peptides (or proteins) bearing foreign donor-type antigenic epitopes in conjunction with concomitant immunosuppressive therapy induces prolonged allograft survival and, in some in-

stances, donor-specific unresponsiveness. Altered peptide ligands have been shown to downregulate TcR-mediated responses by incomplete receptor protein phosphorylation, ZAP-70 activation, and lymphokine synthesis [36, 52]. Although it was originally proposed that the decamer-peptide preparation (Allotrap, Sangstad, USA) derived from the α_1 helix of the class I MHC antigen HLA-B27 produced immunosuppression via a selective interaction with host TcRs, it is now believed that the preparation exerts nonspecific effects independent of the TcR pathway, by inhibiting natural killer cell cytotoxicity both in rodent models and in humans [61]. However, the phase II clinical trials of de novo Allotrap therapy failed to document that the preparation protects patients from experiencing rejection episodes. In an alternate approach to the induction of tolerance, Sayegh et al. [49] documented the tolerogenic activities of class II-derived peptides delivered intrathymically or orally in animal models. Our own approach seeks to induce tolerance by genetically engineering tolerogenic class I MHC proteins. Pilot experiments in rats have documented the presence of a cryptic tolerogenic epitope in the α_1 helical region of rat RT1-A molecules [17]. Administration of the engineered molecule via the portal vein route, in conjunction with a brief (7-day) course of CyA, routinely induces tolerance; indeed, the engineered molecule alone can achieve tolerance in a substantial proportion of hosts.

Conclusion

Advances in the practice of transplantation are integrally related to developments in the field of immunosuppression. During the first 70 years of investigation, radiation and cytotoxic and cytostatic chemicals were used to produce nonselective immunosuppression. The greatest advance during the past decade was the development of the immunoregulatory, T-cell-selective agents CyA, OKT3, TRL, and SRL. However, when used individually, these agents have narrow therapeutic windows. In the 1990s, the administration of combinations of subtherapeutic doses of CyA and SRL, drugs that act both selectively and synergistically, has reduced the incidence of acute rejection episodes substantially and mitigated the need for chronic corticosteroid therapy. However, because the CyA/SRL combination shows potentially overlapping toxicity profiles, pharmacokinetic interactions, and limited immunological activity in rigorous rejection models, further research may be required to discover regimens that more effectively exploit the immunosuppressive strategy of synergism. During the next millennium, the primary goal of research in immunosuppression must be the development of a method to induce specific immunological tolerance of clonotypic lymphoid cells directed against foreign donor epitopes without affecting the rest of the immune repertoire. Thus, just as 3000 years ago the actions of the three Fates established the rationale by which people conducted their lives, the coalescence of three effects – selectivity, synergy, and specificity – will undoubtedly be the basis of the immunosuppressive regimen of the future.

Acknowledgements The author is indebted to Ms. Jennifer Senft for editorial assistance and to Mr. Mike De La Flor for the artistic rendition of the figure. This work was supported by a grant (NIDDK 38016-09) from the National Institute of Diabetes, Digestive and Kidney Diseases.

References

1. Adachi K, Kohara T, Nakao N, Arita M, Chiba K, Mishina T, Sasaki S, Fujita T (1995) Design, synthesis, and structure-activity relationships of 2-substituted-2-amino-1, 3-propanediols: discovery of a novel immunosuppressant, FTY720. Bioorganic & Medical Chemistry Letters 5: 853–856
2. Batiuk TD, Pazderka F, Enns J, DeCastro L, Halloran PF (1995) Calcineurin activity is only partially inhibited in leukocytes of cyclosporine-treated patients. Transplantation 59: 1400–1404
3. Bijsterbosch MK, Meade CJ, Turner GA, Klaus GG (1985) B lymphocyte receptors and polyphosphoinositide degradation. Cell 41: 999–1006
4. Brown EJ, Beal PA, Keith CT, Chen J, Shin TB, Schreiber SL (1995) Control of p70 S6 kinase by kinase activity of FRAP in vivo. Nature 377: 441–446
5. Burkly L, Mulrey N, Blumenthal R, Dimitrov DS (1995) Synergistic inhibition of human immunodeficiency virus type 1 envelope glycoprotein-mediated cell fusion and infection by an antibody to CD4 domain 2 in combination with anti-gp120 antibodies. J Virol 69: 4267–4273
6. Calne RY, Alexandre GP, Murray JE (1962) A study of the effects of drugs in prolonged survival of homologous renal transplantation in dogs. Ann N Y Acad Sci 99: 743–761
7. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Pentlow BD, Rolles K (1978) Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet II: 1323–1327
8. Calne RY, Collier DS, Lim S (1989) Rapamycin for immunosuppression in organ allografting. Lancet II: 227
9. Calvo V, Crews CM, Vik TA, Bierer BE (1992) Interleukin-2 stimulation of p70 S6. Proc Natl Acad Sci USA 89: 7571–7575
10. Cameron AM, Steiner JP, Roskams AJ, Ali SM, Ronnett GV, Snyder SH (1995) Calcineurin associated with the insoitol 1,4,5-triphosphate receptor-FKBP12 complex modulates Ca2+ flux. Cell 83: 463–472

11. Charleton B, Auchincloss H Jr, Fathman CG (1994) Mechanisms of transplantation tolerance. Ann Rev Immunol 12: 797–734
12. Chou T-C, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22: 27–55
13. Chung J, Kuo CJ, Crabtree GR, Blenis J (1992) Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell 69: 1227–1236
14. Cifone MG, De Maria R, Roncaioli P, Rippo MR, Azuma M, Lanier LL, Sàntoni A, Testi R (1994) Apoptosis signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. J Exp Med 177: 1547–1552
15. Cosimi AB, Colvin RB, Burton RC, Rubin RH, Goldstein G, Kung PC, Hansen WP, Delmonico FL, Russell PS (1981) Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. N Engl J Med 305: 308–314
16. Garver TD, Kincaid RL, Billingsley ML (1996) In vivo reduction of calcineurin activity in brain by antisense oligonucleotides leads to persistent phosphorylation of tau protein at Thr-181 and Thr-182. J Biol Chem (in press)
17. Ghobrial R, Hamashima T, Stepkowski SM, Kahan BD (1996) Induction of transplantation tolerance by chimeric donor/recipient class I RT1.A^a molecules. Transplantation (in press)
18. Griffith JP, Kim JL, Kim EE, Sintchak MD, Thomson JA, Fitzgibbon MJ, Fleming MA, Caron PR, Hsiao K, Navia MA (1995) X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12-FK506 complex. Cell 82: 507–522
19. Hamburger J, Vayesse J, Crosnier J (1995) Transplantation of kidney between nonmonozygotic twins after irradiation of the recipient. Presse Med 67: 1771–1775
20. Hannun YA (1994) The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 269: 3125–3128
21. Haug CE, Colvin RB, Delmonico FL, Auchincloss H Jr, Tolkoff-Rubin N, Preffer FI, Rothlein R, Norris S, Scharschmidt L, Cosimi AB (1993) A phase I trial of immunosuppression with anti-ICAM-1 (CD54) mAb in renal allograft recipients. Transplantation 55: 766–772
22. Hitchings GH (1973) Biochemical background of trimethoprin-sulphamethoxazole. Med J Aust 1: 5–9
23. Hitchings GH, Elion GB (1954) The chemistry and biochemistry of purine analogs. Ann N Y Acad Sci 60: 195
24. Holman E (1924) Protein sensitization in iso skin grafting: is the latter of practical value? Surg Gynecol Obstet 38: 100
25. Jensen CO (1903) Transplantation of mammary gland carcinoma in mice. Zentralbl Bakteriol 34: 28
26. Kahan BD (1989) Cyclosporine. N Engl J Med 321: 1725–1738
27. Kahan BD, Gibbons S, Tejpal N, Stepkowski SM, Chou T-C (1991) Synergistic interactions of cyclosporine and rapamycin to inhibit immune performances of normal human peripheral blood lymphocytes in vitro. Transplantation 51: 232–239
28. Kahan BD, Tejpal N, Gibbons-Stubbers S, Tu Z, Wang M, Stepkowski SM, Chou TC (1993) The synergistic interactions in vitro and in vivo of brequinar sodium with cyclosporine or rapamycin alone and in triple combination. Transplantation 55: 894–900
29. Kaplan JC, Hirsch MS (1994) Therapy other than reverse transcriptase inhibitors for HIV infection. Clin Lab Med 14: 367–391
30. Kelly KJ, Williams WW, Colvin RB, Bonventre JV (1994) Antibody to intercellular adhesion molecule-1 protects the kidney against ischemic injury. Proc Natl Acad Sci USA 91: 812–816
31. Knight RJ, Kurrle R, Stepkowski SM, Serino F, Chou TC, Kahan BD (1994) Synergistic immunosuppressive actions of cyclosporine with a mouse anti-rat α/β-T cell receptor monoclonal antibody. Transplantation 57: 1544–1548
32. Kolesnick RN, Golde DW (1994) The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 77: 325–328
33. Koyama H, Tsuji M (1983) Genetic and biochemical studies on the activation and cytotoxic mechanism of bredinin, a potent inhibitor of purine biosynthesis in mammalian cells. Biochem Pharmacol 32: 3547–3553
34. Lindholm A, Kahan BD (1993) Influence of cyclosporine pharmacokinetic parameters, trough concentrations and AUC monitoring on outcome after kidney transplantation. Clin Pharmacol Ther 54: 205–218
35. Liu J, Farmer JD, Lane WS, Friedman J, Weissman I, Schreiber SL (1991) Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 66: 807–815
36. Madrenas J, Wange RL, Wang JL, Isakov N, Samelson LE, Germain RN (1995) Zeta phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. Science 267: 515–518
37. Medawar PB (1944) The behavior and fate of skin autografts and skin homografts in rabbits. J Anat 78: 176
38. Milan D, Griffith J, Su M, Price ER, McKeon F (1994) The latch region of calcineurin B is involved in both immunosuppressant-immunophilin complex docking and phosphatase activation. Cell 79: 437–447
39. Morice WG, Wiederrecht G, Brunn GJ, Siekierka JJ, Abraham RT (1993) Rapamycin inhibition of interleukin-2 dependent $p33^{cdk2}$ and $p34^{cdc2}$ kinase activation in T lymphocytes. J Biol Chem 268: 22737–22745
40. Murgia MG, Jordan S, Kahan BD (1996) The side effect profile of sirolimus: a phase I study in quiescent cyclosporine-prednisone-treated renal transplant patients. Kidney Int 49: 209–216
41. Murray JE, Merrill LP, Harrison JH, Wilson RE, Dammin GJ (1962) Prolonged survival of human kidney homografts by immunosuppressive drug therapy. N Engl J Med 268: 1315–1323
42. Ochiai T, Nagata M, Nakajima K, et al (1987) Studies of the effects of FK506 on renal allografting in the beagle dog. Transplantation 44: 729–733
43. Platz KP, Sollinger HW, Hullett DA (1991) RS-61443. A new potent immunosuppressive agent. Transplantation 51: 27–31
44. Rideout DC, Bustamante A, Patel R, Henderson GB (1995) Suramin sodium: pronounced effects on methotrexate transport and anti-folate activity in cultured tumor cells. Int J Cancer 61: 840–847
45. Rothlein R, Dustin ML, Marlin SD, Springer TA (1986) A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137: 1270–1274
46. Russell BRG (1912) The manifestation of active resistance to the growth of implanted cancer. Fifth Sci Rep Cancer Res Fund: 1
47. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH (1994) RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 78: 35–43

48. Sabers CJ, Martin MM, Brunn GJ, Williams JM, Dumont FJ, Wiederracht G, Abraham RT (1995) Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. J Biol Chem 270: 815–822
49. Sayegh MH, Perico N, Gallon L, Imberti O, Hancock WW, Remuzzi G, Carpenter C (1994) Mechanisms of acquired thymic unresponsiveness to renal allografts: thymic recognition of immunodominant allo-MHC peptides induces peripheral T cell anergy. Transplantation 58: 125–132
50. Schwartz RS, Dameshek W (1959) Drug-induced immunological tolerance. Nature 183: 1682
51. Slaton JW, Kahan BD (1996) Case report – Sirolimus rescue therapy for refractory renal allograft rejection. Transplantation 61: 977–979
52. Sloan-Lancaster J, Evavold BD, Allen PM (1993) Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. Nature 363: 156–159
53. Stepkowski SM, Chen H, Daloze P, Kahan BD (1991) Rapaymcin, a potent immunosuppressive drug for vascularized heart, kidney, and small bowel transplantation in the rat. Transplantation 51: 22–26
54. Stepkowski SM, Chen H-F, Wang M-E, Daloze P, Kahan BD (1992) Inhibition of host-versus-graft and graft-versus-host responses after small bowel transplantation in rats by rapamycin. Transplantation 53: 258–264
55. Stepkowksi SM, Tu Y, Condon TP, Bennett CF (1994) Blocking of heart allograft rejection by intercellular adhesion molecule-1 antisense oligonucleotides alone or in combination with other immunosuppressive modalities. J Immunol 153: 5336–5346
56. Stepkowski SM, Napoli KL, Wang M-E, Qu X, Chou T-C, Kahan BD (1996) The effects of the pharmacokinetic interaction between orally administered sirolimus and cyclosporine on the synergistic prolongation of heart allograft survival in rats. Transplantation (in press)
57. Suzuki S, Enosawa S, Kakefuda T, Shinomiya T, Amari M, Naoe S, Hoshino Y, Chiba K (1996) A novel immunosuppressant, FTY720, with a unique mechanism of action, induces long-term graft acceptance in rat and dog allotransplantation. Transplantation 61: 200–205
58. Tu Y, Stepkowski SM, Chou T-C, Kahan BD (1995) The synergistic effects of cyclosporine, sirolimus, and brequinar on heart allograft survival in mice. Transplantation 59: 177–183
59. Vathsala A, Chou T-C, Kahan BD (1990) Analysis of the interactions of immunosuppressive drugs with cyclosporine in inhibiting DNA proliferation. Transplantation 49: 463–472
60. Wang M-E, Stepkowski SM, Ferraresso M, Kahan BD (1992) Evidence that rapamycin rescue therapy delays rejection of major (MHC) plus minor (non-MHC) histoincompatible heart allografts in rats. Transplantation 54: 704–709
61. Woo J, Gao L, Cornejo MC, Buelow R (1995) A synthetic dimeric HLA class I peptide inhibits T cell activity in vitro and prolongs allogeneic heart graft survival in a mouse model. Transplantation 60: 1156–1163