REVIEW ARTICLE

DRUG THERAPY

Immunosuppressive Drugs for Kidney Transplantation

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THE CENTRAL ISSUE IN ORGAN TRANSPLANTATION REMAINS SUPPRESsion of allograft rejection. Thus, development of immunosuppressive drugs is the key to successful allograft function. Immunosuppressive agents are used for induction (intense immunosuppression in the initial days after transplantation), maintenance, and reversal of established rejection. This review focuses on agents that are either approved or in phase 2 or phase 3 trials in kidney transplantation, but many issues covered here are applicable to all organ transplantation. I begin with a model of the alloimmune response to illustrate how these medications act.

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THREE-SIGNAL MODEL OF ALLOIMMUNE RESPONSES

Alloimmune responses involve both naive and memory lymphocytes,[1] including lymphocytes previously stimulated by viral antigens cross-reacting with HLA antigens.[2] In the graft and the surrounding tissues, dendritic cells of donor and host origin become activated and move to T-cell areas of secondary lymphoid organs. There, antigen-bearing dendritic cells engage alloantigen-reactive naive T cells and central memory T cells that recirculate between lymphoid compartments but cannot enter peripheral tissues[3] (Fig. 1). Naive T cells are optimally triggered by dendritic cells in secondary lymphoid organs,[6,7] but antigen-experienced cells may be activated by other cell types, such as graft endothelium.[8]

An antigen on the surface of dendritic cells that triggers T cells with cognate T-cell receptors constitutes "signal 1," transduced through the CD3 complex. Dendritic cells provide costimulation, or "signal 2," delivered when CD80 and CD86 on the surface of dendritic cells engage CD28 on T cells. Signals 1 and 2 activate three signal transduction pathways: the calcium–calcineurin pathway, the RAS–mitogen-activated protein (MAP) kinase pathway, and the nuclear factor-κB pathway.[9] These pathways activate transcription factors that trigger the expression of many new molecules, including interleukin-2, CD154, and CD25. Interleukin-2 and other cytokines (e.g., interleukin-15) activate the "target of rapamycin" pathway to provide "signal 3," the trigger for cell proliferation. Lymphocyte proliferation also requires nucleotide synthesis. Proliferation and differentiation lead to a large number of effector T cells. B cells are activated when antigen engages their antigen receptors, usually in lymphoid follicles or in extrafollicular sites, such as red pulp of spleen,[10] or possibly in the transplant,[11] producing alloantibody against donor HLA antigens. Thus, within days the immune response generates the agents of allograft rejection, effector T cells and alloantibody.

EFFECTORS AND LESIONS OF REJECTION

Effector T cells that emerge from lymphoid organs infiltrate the graft and orchestrate an inflammatory response. In T-cell–mediated rejection, the graft is infiltrated by effec-

tor T cells, activated macrophages, B cells, and plasma cells and displays interferon-γ effects, increased chemokine expression, altered capillary permeability and extracellular matrix, and deterioration of parenchymal function. The diagnostic lesions of T-cell–mediated rejection reflect mononuclear cells invading the kidney tubules (tubulitis) and the intima of small arteries (arteritis). Macrophages that are activated by T cells participate through delayed-type hypersensitivity,[12] but the injury remains antigen-specific.[13] Injury is not simply lysis of target cells, since typical lesions develop in mice lacking cytotoxic T-cell lytic molecules,[14] but may involve parenchymal transdifferentiation into mesenchymal cells[15] and cell senescence.[16]

Alloantibody against donor antigens that is produced systemically or locally in the graft targets capillary endothelium.[17] Antibody-mediated rejection is diagnosed by clinical,[18] immunologic,[19] and histologic criteria, including a demonstration of complement factor C4d in capillaries.[20]

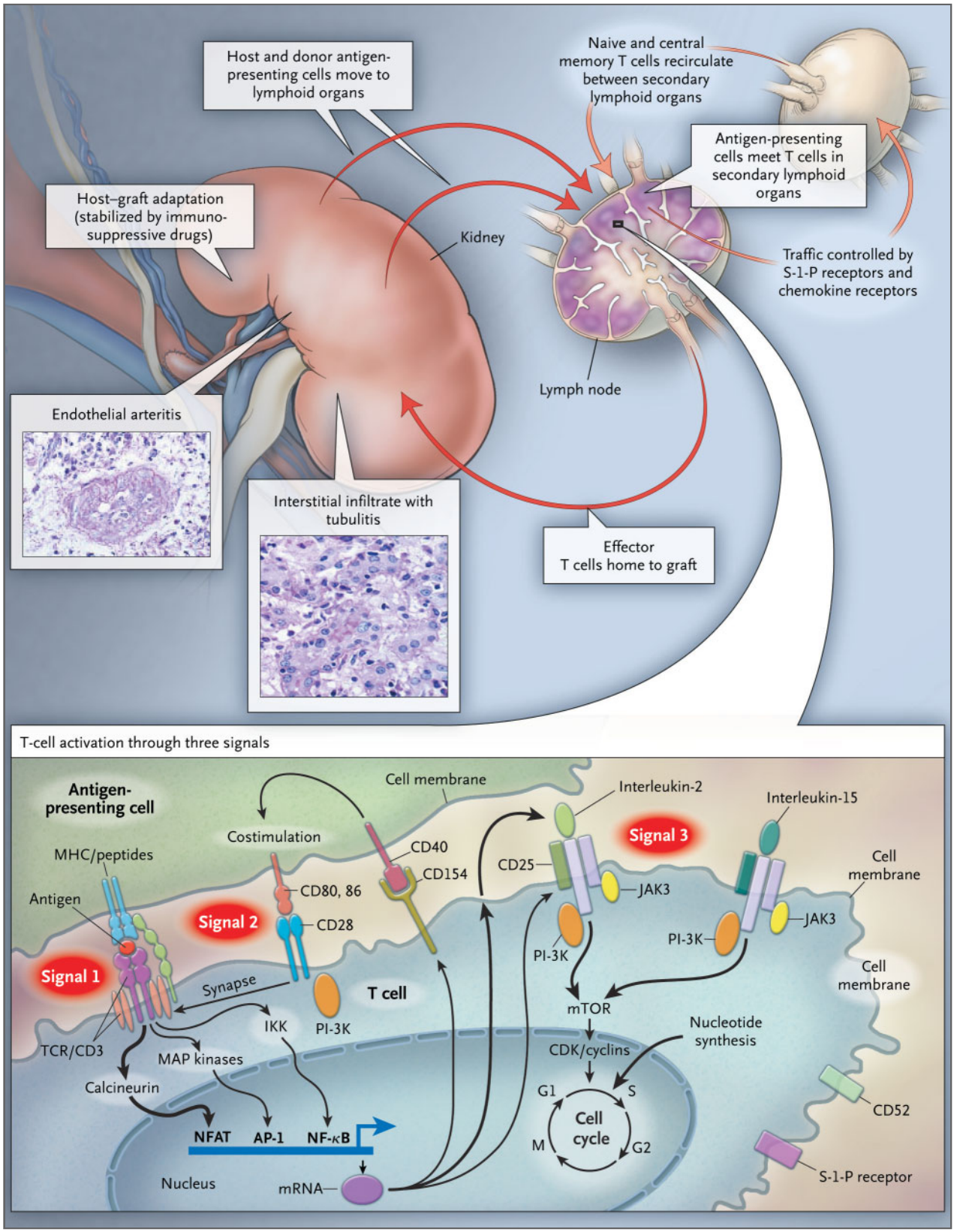
HOST–GRAFT ADAPTATION

The term "host–graft adaptation" describes the decrease in both donor-specific responsiveness and the risk of rejection in the months after a successful transplantation that is maintained with immunosuppression.[21] Changes in the organ — a loss of donor dendritic cells and a resolution of injury — contribute to the adaptation. Regulatory T cells may also be able to control alloimmune responses, by analogy with their ability to suppress autoimmunity,[22] although this hypothesis is unproven. The crucial element is that host T cells become less responsive to donor antigens when antigen persists and immunosuppression is maintained. This may be a general characteristic of T-cell responses in vivo, in which antigen persistence with inadequate costimulation triggers adaptations that limit T-cell responsiveness.[23] The resulting partial T-cell anergy (known as "adaptive tolerance" or "in vivo anergy") is characterized by decreased tyrosine kinase activation and calcium mobilization (signal 1) and decreased response to interleukin-2 (signal 3). Adaptation in clinical transplantation resembles in vivo anergy — for example, both can occur in the presence of calcineurin inhibitors. The role of maintenance immunosuppression may be to stabilize adaptation by limiting excitation of the immune system and thus antigen presentation. In some experimental models, favorable adaptations are blocked when calcineurin is inhibited,[24] leading to suggestions that calcineurin inhibitors prevent adaptations in clinical transplantation. However, the relevance of these models to clinical adaptation, which occurs despite treatment with calcineurin inhibitors, is doubtful.

Figure 1 (facing page). Steps in T-Cell–Mediated Rejection.
Antigen-presenting cells of host or donor origin migrate to T-cell areas of secondary lymphoid organs. These T cells ordinarily circulate between lymphoid tissues, regulated by chemokine and sphingosine-1-phosphate (S-1-P) receptors.[4] APCs present donor antigen to naive and central memory T cells. Some presentation of antigen by donor cells in the graft cannot be excluded (e.g., endothelial cells that activate antigen-experienced T cells). T cells are activated and undergo clonal expansion and differentiation to express effector functions. Antigen triggers T-cell receptors (TCRs) (signal 1) and synapse formation.[5] CD80 (B7-1) and CD86 (B7-2) on the APC engage CD28 on the T cell to provide signal 2. These signals activate three signal-transduction pathways — the calcium–calcineurin pathway, the mitogen-activated protein (MAP) kinase pathway, and the protein kinase C–nuclear factor-κB (NF-κB) pathway — which activate transcription factors nuclear factor of activated T cells (NFAT), activating protein 1 (AP-1), and NF-κB, respectively. The result is expression of CD154 (which further activates APCs), interleukin-2 receptor α chain (CD25), and interleukin-2. Receptors for a number of cytokines (interleukin-2, 4, 7, 15, and 21) share the common γ chain, which binds Janus kinase 3 (JAK3). Interleukin-2 and interleukin-15 deliver growth signals (signal 3) through the phosphoinositide-3-kinase (PI-3K) pathway and the molecular-target-of-rapamycin (mTOR) pathway, which initiates the cell cycle. Lymphocytes require synthesis of purine and pyrimidine nucleotides for replication, regulated by inosine monophosphate dehydrogenase (IMPDH) and dihydroorotate dehydrogenase (DHODH), respectively. Antigen-experienced T cells home to and infiltrate the graft and engage the parenchyma to create typical rejection lesions such as tubulitis and, in more advanced rejection, endothelial arteritis. However, if the rejection does not destroy the graft, adaptation occurs and is stabilized by immunosuppressive drugs. The photomicrographs of tubulitis and endothelial arteritis are taken from a mouse model in which these lesions are T-cell–dependent but independent of perforin, granzymes, and antibody. IKK denotes inhibitor of nuclear factor-κB kinase , CDK cyclin-dependent kinase, and MHC major histocompatibility complex.

IMMUNOSUPPRESSIVE DRUGS

Immunosuppression can be achieved by depleting lymphocytes, diverting lymphocyte traffic, or blocking lymphocyte response pathways (Fig. 2). Immunosuppressive drugs have three effects: the therapeutic effect (suppressing rejection), unde-



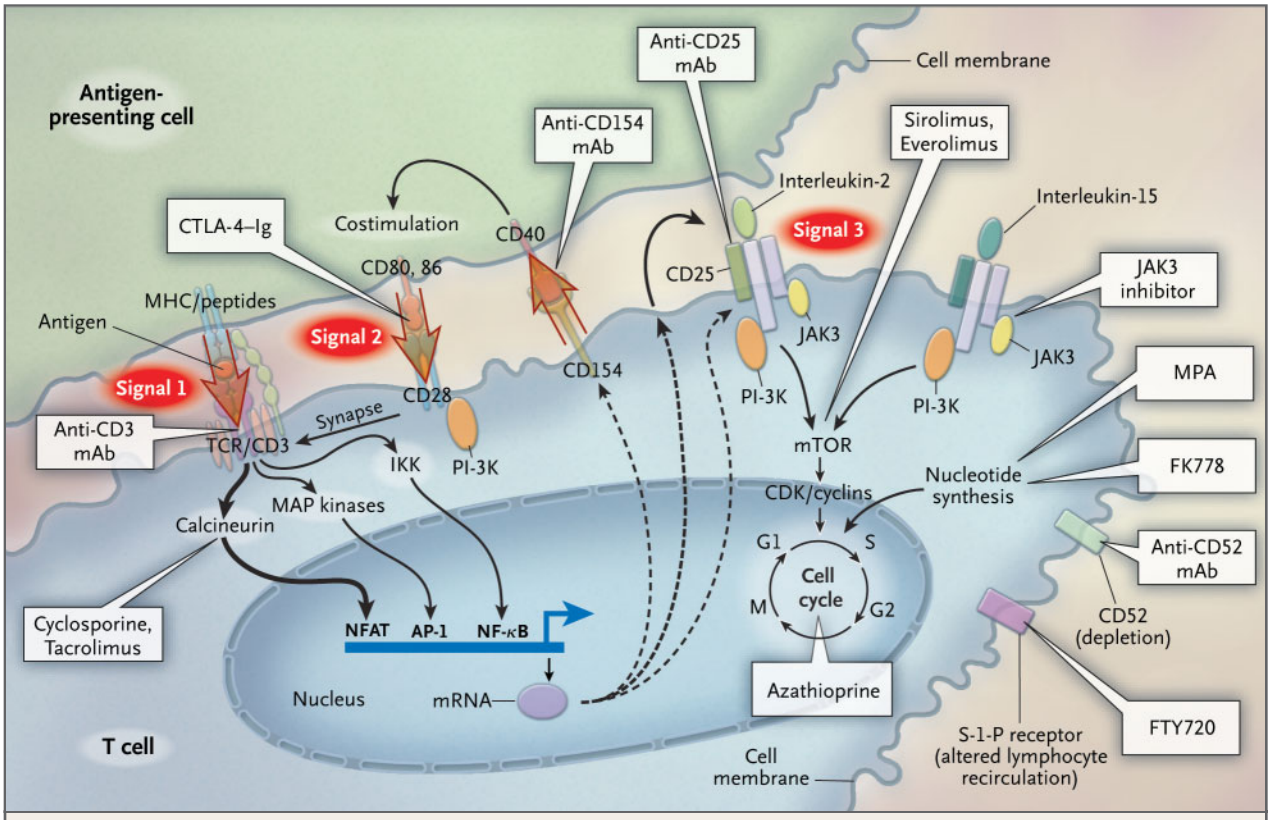


Figure 2. Individual Immunosuppressive Drugs and Sites of Action in the Three-Signal Model.

Anti-CD154 antibody has been withdrawn from clinical trials but remains of interest. FTY720 engagement of sphingosine-1-phosphate (S-1-P) receptors triggers and internalizes the receptors and alters lymphocyte recirculation, causing lymphopenia. Antagonists of chemokine receptors (not shown) are also being developed in preclinical models. MPA denotes mycophenolic acid.

sired consequences of immunodeficiency (infection or cancer), and nonimmune toxicity to other tissues. Immunodeficiency leads to characteristic infections and cancers, such as post-transplantation lymphoproliferative disease,[25] which are related more to the intensity of immunosuppression than to the specific agent used.

New immunosuppressive protocols underscored this point by evoking a new infectious complication, BK-related polyomavirus nephropathy.[26] This syndrome of tubular injury by a virus that is usually innocuous emerged only with the recent introduction of powerful drug combinations and now contributes to renal injury and graft loss. Fortunately, the newer immunosuppressive agents have resulted in a lower incidence of both infection and cancer than might have been expected, perhaps because preventing rejection reduces the need for powerful agents to reverse it.

Nonimmune toxicity is agent-specific and is often related to the mechanism that is used, because each agent or class of drugs targets molecules with physiologic roles in nonimmune tissues. For example, nephrotoxicity of calcineurin inhibitors may reflect a role of calcineurin within the renal vasculature.

CLASSIFICATION OF IMMUNOSUPPRESSIVE DRUGS

Immunosuppressive drugs include small-molecule drugs, depleting and nondepleting protein drugs (polyclonal and monoclonal antibodies), fusion proteins, intravenous immune globulin, and glucocorticoids (Table 1). Because of space limitations, intravenous immune globulin and glucocorticoids cannot be discussed in detail. In brief, intravenous immune globulin has multiple effects[27] and is an important component of approaches to suppress alloantibody responses. Glucocorticoids act as agonists of glucocorticoid receptors, but at higher dos-

es they have receptor-independent effects. Receptor-mediated effects are mainly transcriptional through DNA-binding and protein–protein interactions of the steroid-receptor complex, targeting transcription factors such as activator protein 1 and nuclear factor-κB.[28]

Most small-molecule immunosuppressive agents are derived from microbial products and target proteins that have been highly conserved in evolution. Small-molecule immunosuppressive drugs at clinically tolerated concentrations probably do not saturate their targets. For example, cyclosporine acts by inhibiting calcineurin but only partially inhibits calcineurin as used clinically.[29] Without target saturation, the drug's effects are proportional to the concentration of the drug, which makes dosing and monitoring critical.

Depleting protein immunosuppressive agents are antibodies that destroy T cells, B cells, or both. T-cell depletion is often accompanied by the release of cytokines, which produces severe systemic symptoms, especially after the first dose. The use of depleting antibodies reduces early rejection but increases the risks of infection and post-transplantation lymphoproliferative disease and can be followed by late rejection as the immune system recovers. Recovery from immune depletion takes months to years and may never be complete in older adults. The depletion of antibody-producing cells is better tolerated than T-cell depletion, because it is not usually accompanied by cytokine release and immunoglobulin levels are usually maintained. However, depletion of antibody-producing cells is incomplete because many plasma cells are resistant to the available antibodies that target B cells, such as anti-CD20 antibody.

Nondepleting protein drugs are monoclonal antibodies or fusion proteins that reduce responsiveness without compromising lymphocyte populations. They typically target a semiredundant mechanism such as CD25, which explains their limited efficacy but the absence of immunodeficiency complications. These drugs have low nonimmune toxicity because they target proteins that are expressed only in immune cells and trigger little release of cytokines.

Table 1. Classification of Immunosuppressive Therapies Used in Organ Transplantation or in Phase 2–3 Trials.*

- Glucocorticoids
- Small-molecule drugs
  - Immunophilin-binding drugs
    - Calcineurin inhibitors
      - Cyclophilin-binding drugs: cyclosporine, ISA(TX)247†
      - FKBP12-binding drugs: tacrolimus, modified-release tacrolimus‡
    - Target-of-rapamycin inhibitors: sirolimus, everolimus
  - Inhibitors of nucleotide synthesis
    - Purine synthesis (IMPDH) inhibitors
      - Mycophenolate mofetil
      - Enteric-coated mycophenolic acid
      - Mizoribine§
    - Pyrimidine synthesis (DHODH) inhibitors
      - Leflunomide¶
      - FK778†
  - Antimetabolites: azathioprine
  - Sphingosine-1-phosphate–receptor antagonists: FTY720‡
- Protein drugs
  - Depleting antibodies (against T cells, B cells, or both)
    - Polyclonal antibody: horse or rabbit antithymocyte globulin
    - Mouse monoclonal anti-CD3 antibody (muromonab-CD3)
    - Humanized monoclonal anti-CD52 antibody (alemtuzumab)¶
    - B-cell–depleting monoclonal anti-CD20 antibody (rituximab)¶
  - Nondepleting antibodies and fusion proteins
    - Humanized or chimeric monoclonal anti-CD25 antibody (daclizumab, basiliximab)
    - Fusion protein with natural binding properties: CTLA-4–Ig (LEA29Y†)
  - Intravenous immune globulin

* FKBP12 denotes FK506-binding protein 12, IMPDH inosine monophosphate dehydrogenase, DHODH dihydroorotate dehydrogenase, and CTLA-4 cytotoxic T-lymphocyte–associated antigen 4.
† This treatment is being used in phase 2 trials in renal transplantation.
‡ This treatment is being used in phase 3 trials in renal transplantation.
§ Mizoribine is being used as an immunosuppressive drug in Japan.
¶ This drug is being evaluated for off-label use as an immunosuppressive agent.

SMALL-MOLECULE DRUGS

Azathioprine, which is derived from 6-mercaptopurine, was the first immunosuppressive agent to achieve widespread use in organ transplantation[30] (Table 2). The developers of azathioprine, Gertrude Elion and George Hitchings, were acknowledged by a share of the 1988 Nobel Prize. Azathioprine is thought to act by releasing 6-mercaptopurine, which interferes with DNA synthesis. Other possible mechanisms include converting costimulation into an apoptotic signal.[41] After cyclosporine was introduced, azathioprine became a second-line drug.

Table 2. Characteristics of Small-Molecule Immunosuppressive Drugs Used in Organ Transplantation or in Phase 2–3 Trials.*

Drug	Description	Mechanism	Nonimmune Toxicity and Comments
Cyclosporine	11-amino-acid cyclic peptide from *Tolypocladium inflatum*[31]	Binds to cyclophilin; complex inhibits calcineurin phosphatase and T-cell activation	Nephrotoxicity, hemolytic–uremic syndrome, hypertension, neurotoxicity, gum hyperplasia, skin changes, hirsutism, post-transplantation diabetes mellitus, hyperlipidemia; trough monitoring or checking levels two hours after administration required
Tacrolimus (FK506)	Macrolide antibiotic from *Streptomyces tsukubaensis*[32,33]	Binds to FKBP12; complex inhibits calcineurin phosphatase and T-cell activation	Effects similar to those of cyclosporine but with a lower incidence of hypertension, hyperlipidemia, skin changes, hirsutism, and gum hyperplasia and a higher incidence of post-transplantation diabetes mellitus and neurotoxicity; trough monitoring required
Sirolimus (rapamycin)	Triene macrolide antibiotic from *S. hygroscopicus* from Easter Island (Rapa Nui)[34]	Binds to FKBP12; complex inhibits target of rapamycin and interleukin-2–driven T-cell proliferation	Hyperlipidemia, increased toxicity of calcineurin inhibitors, thrombocytopenia, delayed wound healing, delayed graft function, mouth ulcers, pneumonitis, interstitial lung disease; lipid monitoring required; recipients whose risk of rejection is low to moderate can stop cyclosporine treatment two to four months after transplantation
Everolimus	Derivative of sirolimus		
Mycophenolate mofetil and enteric-coated mycophenolate	Mycophenolic acid from penicillium molds[35-37]	Inhibits synthesis of guanosine monophosphate nucleotides; blocks purine synthesis, preventing proliferation of T and B cells	Gastrointestinal symptoms (mainly diarrhea), neutropenia, mild anemia; blood-level monitoring not required but may improve efficacy; absorption reduced by cyclosporine
FK778 and malononitrilamide	Modification of A77 1726 (active derivative of leflunomide)	Inhibits pyrimidine synthesis, blocking proliferation of T and B cells	Anemia; other effects not known; in phase 2 trials
Azathioprine	Prodrug that releases 6-mercaptopurine	Converts 6-mercaptopurine to tissue inhibitor of metalloproteinase, which is converted to thioguanine nucleotides that interfere with DNA synthesis; thioguanine derivatives may inhibit purine synthesis	Leukopenia, bone marrow depression, macrocytosis, liver toxicity (uncommon); blood-count monitoring required
FTY720	Sphingosine-like derivative of myriocin from ascomycete fungus[38]	Works as an antagonist for sphingosine-1-phosphate receptors on lymphocytes, enhancing homing to lymphoid tissues and preventing egress, causing lymphopenia	Reversible first-dose bradycardia, potentiated by general anesthetics and beta-blockers; nausea, vomiting, diarrhea, increased liver-enzyme levels
CP-690,550[39]; and Tyrphostin AG 490[40]	Synthetic molecule	Binds cytoplasmic tyrosine kinase JAK3, inhibiting cytokine-induced signaling	Anemia caused by potential effects on JAK2

* Data about drugs come from the manufacturer's inserts for health care professionals unless otherwise indicated.

Calcineurin Inhibitors

Cyclosporine, a cornerstone of immunosuppression in transplantation for two decades, is in effect a prodrug that engages cyclophilin, an intracellular protein of the immunophilin family, forming a complex that then engages calcineurin.[42] The adverse effects of cyclosporine, which are related to the concentration of the drug, include nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism, and tremor. Cyclosporine can also induce the hemolytic–uremic syndrome and post-transplantation diabetes mellitus. Recent developments include monitoring of the peak cyclosporine levels two hours after administration to better reflect exposure to the drug.[43,44] A chemically modified cyclosporine, ISA(TX)247, is under development.[45]

Tacrolimus engages another immunophilin, FK506-binding protein 12 (FKBP12), to create a complex that inhibits calcineurin with greater molar potency than does cyclosporine. Initial trials in-

dicated that there was less rejection with tacrolimus than with cyclosporine,[46,47] but recent analyses suggest that in the current dosing strategies, the efficacy of cyclosporine is similar to that of tacrolimus.[48,49] Tacrolimus resembles cyclosporine in that it can result in nephrotoxicity and the hemolytic–uremic syndrome, but it is less likely to cause hyperlipidemia, hypertension, and cosmetic problems and more likely to induce post-transplantation diabetes. Tacrolimus has been suspected of inducing more BK-related polyomavirus nephropathy than has cyclosporine in patients who have undergone kidney transplantation, especially when used with mycophenolate mofetil, but renal function may be better with tacrolimus.[49] New developments include a preparation of modified-release tacrolimus to permit once-daily dosing.

The use of tacrolimus has increased steadily, and the drug is now the dominant calcineurin inhibitor, but most transplantation programs exploit the strengths of both tacrolimus and cyclosporine, depending on the risks in individual patients. Hypertension, hyperlipidemia, and the risk of rejection argue for tacrolimus, whereas a high risk of diabetes (e.g., older age or obesity) argues for cyclosporine.

Inosine Monophosphate Dehydrogenase Inhibitors

The use of inhibitors of purine synthesis for immunosuppression was based on the observation that inborn errors in this pathway produce immunodeficiency without damaging other organs, in contrast to errors in the purine salvage pathway.[50,51] Mycophenolic acid inhibits inosine monophosphate dehydrogenase, a key enzyme in purine synthesis. Mycophenolate mofetil is a prodrug that releases mycophenolic acid, and in large-scale trials with cyclosporine, it was superior to azathioprine in preventing rejection of kidney transplants.[52-55] Protocols using mycophenolate mofetil and calcineurin inhibitors improved patient survival and graft survival and reduced early and late allograft rejection.[56,57] Mycophenolate mofetil has also been evaluated in heart transplantation.[58] The drug has largely replaced azathioprine and is widely used because it is effective in combination with many other agents, simple to use without monitoring, and free from organ toxicity and cardiovascular risk. Its principal nonimmune toxic effects are gastrointestinal (mainly diarrhea) and hematologic (anemia, leukopenia). Mycophenolate mofetil may increase cytomegalovirus disease but in vitro manifests antipneumocystis activity.[59] Enteric-coated mycophenolic acid has been introduced as an alternative to mycophenolate mofetil.[60]

Target-of-Rapamycin Inhibitors

Sirolimus[61] and everolimus engage FKBP12 to create complexes that engage and inhibit the target of rapamycin but cannot inhibit calcineurin (Fig. 2). Inhibition of the target of rapamycin blocks signal 3 by preventing cytokine receptors from activating the cell cycle. The principal nonimmune toxic effects of sirolimus and everolimus include hyperlipidemia, thrombocytopenia, and impaired wound healing. Other reported effects include delayed recovery from acute tubular necrosis in kidney transplants, reduced testosterone concentrations,[62] aggravation of proteinuria, mouth ulcers, skin lesions, and pneumonitis. However, sirolimus and everolimus may reduce cytomegalovirus disease.[63] Sirolimus and everolimus were developed for use with cyclosporine,[64,65] but the combination increased nephrotoxicity, the hemolytic–uremic syndrome, and hypertension. Sirolimus has been combined with tacrolimus (e.g., the Edmonton protocol for pancreatic islet transplantation) to avoid the toxicity of sirolimus–cyclosporine combinations.[66,67] However, a controlled trial in renal transplantation showed that sirolimus plus tacrolimus produced more renal dysfunction and hypertension than did mycophenolate mofetil plus tacrolimus,[68] which indicates that sirolimus potentiates tacrolimus nephrotoxicity. Practitioners can reduce the toxicity of the combination of a target-of-rapamycin inhibitor and a calcineurin inhibitor by withdrawing one of the drugs. For example, withdrawing cyclosporine in patients in stable condition who are receiving the sirolimus–cyclosporine combination reduces renal dysfunction and hypertension, with a small increase in rejection episodes,[69] which suggests a strategy for avoiding the toxic effects of calcineurin inhibitors (Table 3).

Sirolimus and everolimus may have antineoplastic and arterial protective effects. Since these agents slow the growth of established experimental tumors,[70] they have potential applications in oncology. The possibility that sirolimus and everolimus can protect arteries is suggested by two observations: target-of-rapamycin inhibitors that are incorporated into coronary stents inhibit restenosis,[71] and target-of-rapamycin inhibitors plus calcineurin inhibitors reduce the incidence of graft coronary artery disease associated with heart trans-

Table 3. Characteristics of Protein Immunosuppressive Drugs Used in Organ Transplantation or in Phase 2–3 Trials.

Drug	Description	Mechanism	Toxicity and Comments*
Polyclonal antithymocyte globulin	Polyclonal IgG from horses or rabbits immunized with human thymocytes; absorbed to reduce unwanted antibodies	Blocks T-cell membrane proteins (CD2, CD3, CD45, and so forth), causing altered function, lysis, and prolonged T-cell depletion	The cytokine-release syndrome (fever, chills, hypotension), thrombocytopenia, leukopenia, serum sickness
Muromonab-CD3	Murine monoclonal antibody against CD3 component of T-cell–receptor signal-transduction complex	Binds to CD3 associated with T-cell receptor, leading to initial activation and cytokine release, followed by blockade of function, lysis, and T-cell depletion	Severe cytokine-release syndrome, pulmonary edema, acute renal failure, gastrointestinal disturbances, changes in central nervous system
Alemtuzumab	Humanized monoclonal antibody against CD52, a 25-to-29-kD membrane protein	Binds to CD52 on all B and T cells, most monocytes, macrophages, and natural killer cells, causing cell lysis and prolonged depletion	Mild cytokine-release syndrome, neutropenia, anemia, idiosyncratic pancytopenia, autoimmune thrombocytopenia, thyroid disease
Rituximab	Chimeric monoclonal antibody against membrane-spanning four-domain protein CD20	Binds to CD20 on B cells and mediates B-cell lysis	Infusion reactions, hypersensitivity reactions (uncommon)
Basiliximab	Chimeric monoclonal antibody against CD25 (interleukin-2–receptor α chain)	Binds to and blocks the interleukin-2–receptor α chain (CD25 antigen) on activated T cells, depleting them and inhibiting interleukin-2–induced T-cell activation	Hypersensitivity reactions (uncommon); two doses required; no monitoring required
Daclizumab	Humanized monoclonal antibody against CD25 (interleukin-2–receptor α chain)	Has similar action to that of basiliximab	Hypersensitivity reactions (uncommon); five doses recommended but two may suffice; no monitoring required
LEA29Y	Protein combining B7-binding portion of CTLA-4 with IgG Fc region	Binds to B7 on T cells, preventing CD28 signaling and signal 2	Effects unknown; in phase 2 trials

* The toxic effects of alemtuzumab, rituximab, and LEA29Y in organ-transplant recipients must be established in phase 3 trials. The toxic effects of alemtuzumab are primarily those reported in nontransplantation trials.

plantation.[63] But alternative explanations exist for both observations. Target-of-rapamycin inhibitors may suppress restenosis of mechanically dilated arteries by suppressing wound healing[72] rather than by atherogenesis and may prevent graft coronary artery disease simply by preventing rejection. Potential arterial protective effects of sirolimus and everolimus must be weighed against the effects of the hyperlipidemia these drugs induce.[73]

Dihydroorotate Dehydrogenase Inhibitors

Dihydroorotate dehydrogenase is a key enzyme in pyrimidine synthesis. Leflunomide is a dihydroorotate dehydrogenase inhibitor that is approved for rheumatoid arthritis but is not widely used in transplantation.[74] Its active metabolite, A77 1726, was modified to create FK778, which is in phase 2 trials in kidney transplantation. FK778 may have activity against BK-related polyomavirus and have a lower incidence of gastrointestinal effects than does mycophenolate mofetil, but its nonimmune toxic effects such as anemia must be evaluated.

FTY720

FTY720 is derived from myriocin, a fungus-derived sphingosine analogue. After phosphorylation, FTY720 engages lymphocyte sphingosine-1-phosphate receptors and profoundly alters lymphocyte traffic, acting as a functional sphingosine-1-phosphate antagonist.[75] FTY720 drives T cells into lymphoid tissues and prevents them from leaving and homing to the graft. Despite low overall toxicity, FTY720 induces reversible bradycardia during the first doses,[76] arousing concern about the potential for cardiac arrest when combined with the influences of other agents (e.g., general anesthetics or beta-blockers). FTY720 in combination with cyclosporine has completed phase 2 trials[77] and entered phase 3 trials in renal transplantation.

DEPLETING ANTIBODIES

Polyclonal antithymocyte globulin is produced by immunizing horses or rabbits with human lymphoid cells, harvesting the IgG, and absorbing out toxic antibodies (e.g., those against platelets and

erythrocytes) (Table 3). As an induction agent, polyclonal antithymocyte globulin is usually used for 3 to 10 days to produce "profound and durable" lymphopenia that lasts beyond one year.[78] In addition to immunodeficiency complications, toxic effects of polyclonal antithymocyte globulin include thrombocytopenia, the cytokine-release syndrome, and occasional serum sickness or allergic reactions. Rabbit preparations of polyclonal antithymocyte globulin (such as Thymoglobulin and ATG-Fresenius) are favored over horse polyclonal antithymocyte globulin because of greater potency.

Muromonab-CD3, a mouse monoclonal antibody against CD3, has been used for 20 years to treat rejection[79] and for induction.[80] Muromonab-CD3 binds to T-cell-receptor–associated CD3 complex and triggers a massive cytokine-release syndrome before both depleting and functionally altering T cells. Humans can make neutralizing antibodies against muromonab-CD3 that terminate its effect and limit its reuse. Prolonged courses of muromonab-CD3 increase the risk of post-transplantation lymphoproliferative disease.[81] The use of muromonab-CD3 declined when newer small-molecule immunosuppressive drugs reduced rejection episodes. A trial of a humanized anti-CD3 monoclonal antibody in kidney transplantation[82] was stopped. (A nonactivating humanized anti-CD3 monoclonal antibody is being developed to suppress beta-cell injury in patients with autoimmune diabetes mellitus of recent onset[83] but is not currently used for transplantation.)

Alemtuzumab, a humanized monoclonal antibody against CD52, massively depletes lymphocyte populations. It is approved for treating refractory B-cell chronic lymphocytic leukemia but is not approved for immunosuppression in transplantation. A small study in renal transplantation that concluded that alemtuzumab induced "prope tolerance" (meaning near-tolerance)[84] was not confirmed in later studies.[85] Predictions that target-of-rapamycin inhibitors plus alemtuzumab would induce tolerance were also not confirmed. This combination is associated with rejection episodes, including antibody-mediated rejection.[86] Side effects of alemtuzumab include first-dose reactions, neutropenia, anemia, and (rarely) pancytopenia and autoimmunity (e.g., hemolytic anemia, thrombocytopenia, and hyperthyroidism).[87] The risks of immunodeficiency complications (infections and cancer) with alemtuzumab are unknown. Alemtuzumab is used off-label for induction in some centers, but controlled trials are needed to establish dosing, safety, and efficacy.

Rituximab (anti-CD20 monoclonal antibody) eliminates most B cells and is approved for treating refractory non-Hodgkin's B-cell lymphomas, including some post-transplantation lymphoproliferative disease in organ-transplant recipients. Rituximab is used off-label in combination with maintenance immunosuppressive drugs, plasmapheresis, and intravenous immune globulin to suppress deleterious alloantibody responses in transplant recipients. Although plasma cells are usually CD20-negative, many are short-lived and require replacement from CD20-positive precursors. Thus, depletion of CD20-positive cells does reduce some antibody responses. CD20-positive B cells can act as secondary antigen-presenting cells, which raises the possibility that rituximab can ameliorate T-cell responses. Off-label applications for rituximab include treatment of antibody-mediated rejection and possibly severe T-cell–mediated rejection[88] and suppression of preformed alloantibody before transplantation. Again, controlled trials are needed.

NONDEPLETING ANTIBODIES AND FUSION PROTEINS

Daclizumab and Basiliximab

The anti-CD25 monoclonal antibodies daclizumab and basiliximab are widely used in transplantation for induction in patients who have a low-to-moderate risk of rejection. Because expression of CD25 (interleukin-2 receptor α chain) requires T-cell activation, anti-CD25 antibody causes little depletion of T cells. Anti-CD25 antibody is moderately effective since it reduces rejection by about one third when used with calcineurin inhibitors and has minimal toxic effects.[89-92]

LEA29Y

LEA29Y is a second-generation cytotoxic-T-lymphocyte–associated antigen 4 (CTLA-4) immune globulin that is a fusion protein combining CTLA-4 (which engages CD80 and CD86) with the Fc portion of IgG. Results of a phase 2 trial in patients undergoing renal transplantation who are receiving mycophenolate mofetil, glucocorticoids, and anti-CD25 antibody are available in abstract form (www.atcmeeting.org/2004). In this trial with six months of follow-up, the effect of repeated administration of LEA29Y was similar to that of cyclosporine in preventing rejection. The LEA29Y trial introduces the concept of long-term use of nondepleting pro-

tein immunosuppressive agents to reduce reliance on toxic small-molecule immunosuppressive drugs.

ADDITIONAL DRUGS

Many of the critical steps that are depicted in Figure 1 can be targeted by small molecules or proteins to create new drugs.[93] Potential targets for small-molecule drugs include those previously discussed (e.g., calcineurin) as well as others (e.g., tyrosine kinases, protein kinase Cθ, MAP kinases such as Jun N-terminal kinase, phosphoinositide-3-kinase, and chemokine receptors). Potential targets for protein drugs include many membrane proteins.

Janus kinase 3 (JAK3) inhibitors[39,40] illustrate how small-molecule immunosuppressive drugs are developed. JAK3, a tyrosine kinase associated with the cytokine receptor γ chain, participates in the signaling of many cytokine receptors (interleukin-2, 4, 7, 9, 15, and 21) (Fig. 1). JAK3 inhibitor CP-690,550[39] was developed by screening a chemical library and modifying candidate compounds to produce an oral agent that is immunosuppressive in rodents and nonhuman primates. One adverse effect is anemia, perhaps reflecting activity against Janus kinase 2, which is needed for erythropoietin action.

PROTOCOL DEVELOPMENT AND EMERGING ISSUES

For two decades, the options for immunosuppressive drugs were initial induction with the use of protein immunosuppressive therapy; preadaptation maintenance therapy with three drugs — a calcineurin inhibitor, a second line of drugs (azathioprine and now mycophenolate mofetil), and glucocorticoids; and postadaptation therapy with the same combination of drugs at lower doses. Rejection was reversed with high-dose steroids or depleting antibodies. Now hundreds of potential combinations exist, and many new protocols have emerged, often including a reduced reliance on glucocorticoids[94] and calcineurin inhibitors. Some examples are listed in Table 4. Developing evidence-based approaches to this confusing choice of protocols presents a challenge.

Progress in the control of early and late rejection and in managing infections such as cytomegalovirus has improved both the survival of patients and the function of grafts.[57,100,101] For example, in kidney transplantation, the estimated glomerular filtration rate has improved[102] and is stable in many patients, rather than slowly deteriorating, as in the past.[57] This raises the hope that many organ transplantations that are performed today represent a permanent cure for end-stage organ failure.

But concerns temper this optimism. Outcomes are not continuing to improve,[103] and the rate of late graft loss remains excessive. For example, in the United States each year, end-stage kidney failure develops in 4500 patients who have undergone kidney transplantation, a finding that highlights transplant failure as a major cause of end-stage renal disease.[104] Patients who have undergone liver transplantation have an excessive recurrence rate of hepatitis; coronary artery disease develops in some patients with transplanted hearts; and bronchiolitis obliterans often develops in patients with transplanted lungs.[105,106] The rate of premature death with functioning allografts remains excessive, in part because of cardiovascular and other complications of immunosuppression.

Nonimmune and immunodeficiency complications of transplant immunosuppression should be reduced. The major nonimmune toxic effects are nephrotoxicity, hypertension, hyperlipidemia, diabetes mellitus, and anemia. Five years after surgery, serious renal injury is present in 7 to 21 percent of patients who have undergone nonrenal transplantation,[107] and end-stage kidney failure develops in many patients. The toxic effect of calcineurin inhibitors is an important contributor to the problem of renal failure. Post-transplantation diabetes mellitus develops after three years in 24 percent of patients who have undergone renal transplantation.[108] Hyperlipidemia[109] and anemia[110] are common and undertreated. Options for reducing toxicity include choosing more selective drugs, avoiding toxic combinations, and maintaining vigilance for toxic effects.

Cancers[111] and infections that are induced by transplantation remain frequent, with infections now exceeding rejection in pediatric transplant recipients.[112] Choosing more selective drugs can reduce these risks. For example, anti-CD25 antibody has little effect on the risk of infection and post-transplantation lymphoproliferative disease.[25] New protocols must emphasize reducing the rates of cancer and infection rather than simply lowering the rate of rejection.

New immunosuppressive drug applications and protocols[113] are emerging without adequate trials to establish dosing, safety, and efficacy. Examples are the regimens of induction with alemtuzumab or

Table 4. Examples of Current and Experimental Immunosuppressive Drug Protocols.

Protocol	Protocol Elements			Comments
	Protein Induction	Preadaptation Maintenance	Postadaptation Maintenance*	
Conventional treatment	Anti-CD25 antibody, polyclonal antithymocyte globulin, or none	Calcineurin inhibitor, mycophenolate mofetil, and prednisone	Calcineurin inhibitor and mycophenolate mofetil; prednisone tapered	Possibly excessive immunosuppression during postadaptation
Conventional treatment with no steroids[95]	Anti-CD25 antibody	Calcineurin inhibitor and mycophenolate mofetil; prednisone only if needed	Calcineurin inhibitor and mycophenolate mofetil; prednisone only if needed	Possible increase in rejection
Conventional treatment with depleting antibodies	Polyclonal antithymocyte globulin	Calcineurin inhibitor, mycophenolate mofetil, and prednisone	Calcineurin inhibitor and mycophenolate mofetil; prednisone tapered	Effects of depletion (e.g., increased incidence of post-transplantation lymphoproliferative disorder), possible late rejection
Sirolimus with cyclosporine withdrawal	Anti-CD25 antibody, polyclonal antithymocyte globulin, or none	Cyclosporine, sirolimus, and prednisone	Sirolimus; prednisone tapered	Early toxicity of cyclosporine–sirolimus combination
Calcineurin-inhibitor avoidance with maintenance sirolimus and mycophenolate mofetil[96,97]	Anti-CD25 antibody, polyclonal antithymocyte globulin, or none	Sirolimus, mycophenolate mofetil, and prednisone	Sirolimus and mycophenolate mofetil; prednisone tapered	Possibly excessive early rejection; no phase 3 trials; possible increase in late rejection
Calcineurin-inhibitor withdrawal with mycophenolate mofetil maintenance[98]	Anti-CD25 antibody, polyclonal antithymocyte globulin, or none	Calcineurin inhibitor, mycophenolate mofetil, and prednisone	Mycophenolate mofetil; prednisone tapered	No phase 3 trials
Alemtuzumab induction[84-86]	Alemtuzumab	Sirolimus, prednisone	Sirolimus; prednisone tapered	Long-term consequences of severe depletion unknown; no controlled trials; possible increase in antibody-mediated rejection
Depletion with minimization of immunosuppressive drugs[99]	Polyclonal antithymocyte globulin	Tacrolimus only if no rejection	Minimal tacrolimus if no rejection	Risk of late rejection as lymphoid system recovers
Maintenance with CTLA-4–Ig and mycophenolate mofetil†	Anti-CD25 antibody	CTLA-4–Ig, mycophenolate mofetil, and prednisone	CTLA-4–Ig, mycophenolate mofetil, and prednisone	Efficacy and safety must be established

* For most protocols, no data are available regarding the relative cost and cost-effectiveness of the treatment and long-term requirements for the administration of prednisone.
† CTLA-4–Ig denotes cytotoxic-T-lymphocyte–associated antigen 4 combined with the Fc portion of immunoglobulin G.

radical minimization of maintenance immunosuppression. Moreover, the quality of transplantation trials is suboptimal.[114] One problem is that the decline in the incidence of rejection, the end point in most trials, now limits the evaluation of new agents.[115] New composite end points could incorporate organ function and drug toxicity or emerging laboratory measurements of immune mechanisms.

Optimizing outcomes requires long-term follow-up by knowledgeable caregivers who recognize and react to changes. Allografts with deteriorating function should not be dismissed as instances of "chronic rejection"; instead, the source of injury should be diagnosed (e.g., rejection that is T-cell–mediated or antibody-mediated, recurrent disease, drug toxicity, or infection).[116] The assumption must be that new deterioration reflects new injury, not an inexorable consequence of an earlier injury. The identification of mechanisms of injury may be rewarded by the arresting of further deterioration.

Robust tests for rejection that is T-cell–mediated or antibody-mediated would change clinical management and clinical trials (e.g., microarray

analysis of gene expression in biopsy specimens).[11] Measurement of immune responses could guide transplantation management in the same way that measurement of disease activity guides other fields (e.g., the measurement of lipid levels in the management of hyperlipidemia).

Interest in suppressing alloantibody responses is growing. Emerging evidence links alloantibody to late graft deterioration,[19] and transplantation is increasingly offered to patients who have previously been excluded by existing alloantibody, including ABO blood-group barriers.[117] Options include the optimization of baseline immunosuppression, the administration of rituximab or intravenous immune globulin, and plasmapheresis, but new strategies are needed.

Pharmacogenomics offers possibilities for individualizing immunosuppression, an important goal with respect to toxic drugs with narrow therapeutic indexes.[118,119] For example, CYP3A (cytochrome P-450-3A) allele CYP3A5*1, which is associated with increased CYP3A5 levels, is present in 70 to 80 percent of blacks but in only 5 to 10 percent of whites.[120] Since CYP3A5 genotyping can be used to predict slower achievement of target tacrolimus levels and earlier rejection,[121] it could help reduce rejection in black patients.

For most patients, no practical method of achieving true tolerance to HLA-incompatible organ transplants is at hand. True tolerance is durable antigen-specific unresponsiveness in an immunocompetent host that is induced by previous exposure to the antigen. The only clinical strategy that currently meets this definition is stem-cell transplantation.[122] The stability of the adaptation usually depends on immunosuppression or damage to the immune tissues. At some point, most immunosuppressive agents are billed as tolerogenic, an assertion that is typically followed by the realization that, among at least some patients, the immunologic tolerance is not durable after withdrawal of the drug therapy and recovery from its effects. Indeed, the first report of an immunosuppressive drug was entitled "Drug-Induced Immunological Tolerance."[123] Many "tolerance trials"[124] in fact use immunosuppression and are probably based on host–graft adaptation. Excellent immunosuppression with long-term clinical surveillance remains the best prospect for achieving the potential of transplantation to restore and maintain health.

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REFERENCES

1. Lombardi G, Sidhu S, Daly M, Batchelor JR, Makgoba W, Lechler RI. Are primary alloresponses truly primary? Int Immunol 1990;2:9-13.
2. Adams AB, Williams MA, Jones TR, et al. Heterologous immunity provides a potent barrier to transplantation tolerance. J Clin Invest 2003;111:1887-95.
3. Von Andrian UH, Mackay CR. T-cell function and migration: two sides of the same coin. N Engl J Med 2000;343:1020-34.
4. Mandala S, Hajdu R, Bergstrom J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 2002;296:346-9.
5. Bromley SK, Iaboni A, Davis SJ, et al. The immunological synapse and CD28-CD80 interactions. Nat Immunol 2001;2:1159-66.
6. Lakkis FG, Arakelov A, Konieczny BT, Inoue Y. Immunologic 'ignorance' of vascularized organ transplants in the absence of secondary lymphoid tissue. Nat Med 2000;6:686-8.
7. Zhou P, Hwang KW, Palucki D, et al. Secondary lymphoid organs are important but not absolutely required for allograft responses. Am J Transplant 2003;3:259-66.
8. Biedermann BC, Pober JS. Human endothelial cells induce and regulate cytolytic T cell differentiation. J Immunol 1998;161:4679-87.
9. Wang D, Matsumoto R, You Y, et al. CD3/CD28 costimulation-induced NF-kappaB activation is mediated by recruitment of protein kinase C-theta, Bcl10, and IkappaB kinase beta to the immunological synapse through CARMA1. Mol Cell Biol 2004;24:164-71.
10. MacLennan IC, Toellner KM, Cunningham AF, et al. Extrafollicular antibody responses. Immunol Rev 2003;194:8-18.
11. Sarwal M, Chua M-S, Kambham N, et al. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. N Engl J Med 2003;349:125-38.
12. Bogman MJ, Dooper IM, van de Winkel JG, et al. Diagnosis of renal allograft rejection by macrophage immunostaining with a CD14 monoclonal antibody, WT14. Lancet 1989;2:235-8.
13. Rosenberg AS, Singer A. Cellular basis of skin allograft rejection: an in vivo model of immune-mediated tissue destruction. Annu Rev Immunol 1992;10:333-58.
14. Halloran PF, Urmson J, Ramassar V, et al. Lesions of T-cell-mediated kidney allograft rejection in mice do not require perforin or granzymes A and B. Am J Transplant 2004;4:705-12.
15. Robertson H, Ali S, McDonnell BJ, Burt AD, Kirby JA. Chronic renal allograft dysfunction: the role of T cell-mediated tubular epithelial to mesenchymal cell transition. J Am Soc Nephrol 2004;15:390-7.
16. Halloran PF, Melk A, Barth C. Rethinking chronic allograft nephropathy: the concept of accelerated senescence. J Am Soc Nephrol 1999;10:167-81.
17. Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria — an addition to the Banff 97 classification of renal allograft rejection. Am J Transplant 2003;3:708-14.
18. Halloran PF, Wadgymar A, Ritchie S, Falk J, Solez K, Srinivasa NS. The significance of the anti-class I antibody response. I. Clinical and pathologic features of anti-class I-mediated rejection. Transplantation 1990;49:85-91.
19. Terasaki PI. Humoral theory of transplantation. Am J Transplant 2003;3:665-73.

20. Feucht HE. Complement C4d in graft capillaries — the missing link in the recognition of humoral alloreactivity. Am J Transplant 2003;3:646-52.
21. Starzl TE, Marchioro TL, Waddell WR. The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. Surg Gynecol Obstet 1963;117:385-95.
22. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004;22:531-62.
23. Schwartz RH. T cell anergy. Annu Rev Immunol 2003;21:305-34.
24. Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. Nat Med 1999;5:1298-302.
25. Opelz G, Dohler B. Lymphomas after solid organ transplantation: a Collaborative Transplant Study report. Am J Transplant 2004;4:222-30.
26. Nickeleit V, Klimkait T, Binet IF, et al. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med 2000; 342:1309-15.
27. Kazatchkine MD, Bellon B, Kaveri SV. Mechanisms of action of intravenous immunoglobulin (IVIG). Mult Scler 2000;6:Suppl 2:S24-S26.
28. Karin M. New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable? Cell 1998;93:487-90.
29. Batiuk TD, Pazderka F, Halloran PF. Calcineurin activity is only partially inhibited in leukocytes of cyclosporine-treated patients. Transplantation 1995;59:1400-4.
30. Elion GB. The George Hitchings and Gertrude Elion Lecture: the pharmacology of azathioprine. Ann N Y Acad Sci 1993;685: 400-7.
31. Borel JF, Feurer C, Gubler HJ, Stahelin H. Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 1976; 6:468-75.
32. Goto T, Kino T, Hatanaka H, et al. Discovery of FK-506, a novel immunosuppressant isolated from streptomyces. J Am Chem Soc 1987;109:5031-3.
33. Kino T, Hatanaka H, Miyata S, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. J Antibiot (Tokyo) 1987;40:1256-65.
34. Morris RE. Rapamycins: antifungal, antitumor, antiproliferative and immunosuppressive macrolides. Transplant Rev 1992; 6:39-87.
35. Gosio B. Ricerche batteriologiche e chimiche sulle alterazion del mais; contributo alletiologia della pellagra. Riv Igiene Sanita Pubblica Ann 1896;7:825-68.
36. Florey HW, Gilliver K, Jennings MA, Sanders AG. Mycophenolic acid: an antibiotic from *Penicillium brevicompactum* diercks. Lancet 1946;1:46-9.
37. Mitsui A, Suzuki S. Immunosuppressive effect of mycophenolic acid. J Antibiot (Tokyo) 1969;22:358-63.
38. Brinkmann V, Lynch KR. FTY720: targeting G-protein-coupled receptors for sphingosine 1-phosphate in transplantation and autoimmunity. Curr Opin Immunol 2002;14: 569-75.
39. Changelian PS, Flanagan ME, Ball DJ, et al. Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. Science 2003;302:875-8.
40. Behbod F, Erwin-Cohen RA, Wang M-E, et al. Concomitant inhibition of Janus kinase 3 and calcineurin-dependent signaling pathways synergistically prolongs the survival of rat heart allografts. J Immunol 2001; 166:3724-32.
41. 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.
42. Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 1992;357: 695-7.
43. Clase CM, Mahalati K, Kiberd BA, et al. Adequate early cyclosporin exposure is critical to prevent renal allograft rejection: patients monitored by absorption profiling. Am J Transplant 2002;2:789-95.
44. Cole E, Maham N, Cardella C, et al. Clinical benefits of neoral C2 monitoring in the long-term management of renal transplant recipients. Transplantation 2003;75:2086-90.
45. Stalder M, Birsan T, Hubble RW, Paniagua RT, Morris RE. In vivo evaluation of the novel calcineurin inhibitor ISATX247 in non-human primates. J Heart Lung Transplant 2003;22:1343-52.
46. The U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994; 331:1110-5.
47. European FK506 Multicentre Liver Study Group. Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. Lancet 1994; 344:423-8.
48. Ahsan N, Johnson C, Gonwa T, et al. Randomized trial of tacrolimus plus mycophenolate mofetil or azathioprine versus cyclosporine oral solution (modified) plus mycophenolate mofetil after cadaveric kidney transplantation: results at 2 years. Transplantation 2001;72:245-50.
49. Meier-Kriesche HU, Kaplan B. Cyclosporine microemulsion and tacrolimus are associated with decreased chronic allograft failure and improved long-term graft survival as compared with Sandimmune. Am J Transplant 2002;2:100-4.
50. Giblett ER, Anderson JE, Cohen F, Pollara B, Meuwissen HJ. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 1972;2: 1067-9.
51. Allison AC, Hovi T, Watts RWE, Webster ADB. Immunological observations on patients with the Lesch-Nyhan syndrome, and on the role of *de-novo* purine synthesis in lymphocyte transformation. Lancet 1975;2: 1179-83.
52. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. Transplant 1995;60:225-32.
53. 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.
54. Halloran PF, Mathew T, Tomlanovich S, Groth C, Hooftman L, Barker C. Mycophenolate mofetil in renal allograft recipients: a pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. Transplant 1997;63:39-47. [Erratum, Transplantation 1997;63:618.]
55. European Mycophenolate Mofetil Cooperative Study Group. Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. Lancet 1995;345: 1321-5.
56. Meier-Kriesche HU, Steffen BJ, Hochberg AM, et al. Long-term use of mycophenolate mofetil is associated with a reduction in the incidence and risk of late rejection. Am J Transplant 2003;3:68-73.
57. Gourishankar S, Hunsicker LG, Jhangri GS, Cockfield SM, Halloran PF. The stability of the glomerular filtration rate after renal transplantation is improving. J Am Soc Nephrol 2003;14:2387-94.
58. Kobashigawa J, Miller L, Renlund D, et al. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Transplantation 1998;66:507-15.
59. Oz HS, Hughes WT. Novel anti-Pneumocystis carinii effects of the immunosuppressant mycophenolate mofetil in contrast to provocative effects of tacrolimus, sirolimus, and dexamethasone. J Infect Dis 1997; 175:901-4.
60. Gabardi S, Tran JL, Clarkson MR. Enteric-coated mycophenolate sodium. Ann Pharmacother 2003;37:1685-93.
61. Vézina C, Kudelski A, Sehgal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo) 1975;28:721-6.
62. Fritsche L, Budde K, Dragun D, Einecke G, Diekmann F, Neumayer HH. Testosterone concentrations and sirolimus in male renal transplant patients. Am J Transplant 2004; 4:130-1.
63. Eisen HJ, Tuzcu EM, Dorent R, et al.

Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. N Engl J Med 2003;349:847-58.
64. MacDonald AS. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. Transplantation 2001;71:271-80.
65. Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. Lancet 2000;356:194-202.
66. McAlister VC, Gao Z, Peltekian K, Domingues J, Mahalati K, MacDonald AS. Sirolimus-tacrolimus combination immunosuppression. Lancet 2000;355:376-7.
67. Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230-8.
68. Gonwa T, Mendez R, Yang HC, Weinstein S, Jensik S, Steinberg S. Randomized trial of tacrolimus in combination with sirolimus or mycophenolate mofetil in kidney transplantation: results at 6 months. Transplant 2003;75:1213-20.
69. Johnson RW, Kreis H, Oberbauer R, Brattstrom C, Claesson K, Eris J. Sirolimus allows early cyclosporine withdrawal in renal transplantation resulting in improved renal function and lower blood pressure. Transplantation 2001;72:777-86.
70. Guba M, von Breitenbuch P, Steinbauer M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 2002;8:128-35.
71. Morice M-C, Serruys PW, Sousa JE, et al. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med 2002; 346:1773-80.
72. Schwartz SM, Reidy MA, de Blois D. Factors important in arterial narrowing. J Hypertens Suppl 1996;14:S71-S81.
73. Blum CB. Effects of sirolimus on lipids in renal allograft recipients: an analysis using the Framingham risk model. Am J Transplant 2002;2:551-9.
74. Hardinger KL, Wang CD, Schnitzler MA, et al. Prospective, pilot, open-label, short-term study of conversion to leflunomide reverses chronic renal allograft dysfunction. Am J Transplant 2002;2:867-71.
75. Brinkmann V, Cyster JG, Hla T. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Am J Transplant 2004;4: 1019-25.
76. Budde K, Schmouder RL, Brunkhorst R, et al. First human trial of FTY720, a novel immunomodulator, in stable renal transplant patients. J Am Soc Nephrol 2002;13: 1073-83.
77. Kahan BD, Karlix JL, Ferguson RM, et al. Pharmacodynamics, pharmacokinetics, and safety of multiple doses of FTY720 in stable renal transplant patients: a multicenter, randomized, placebo-controlled, phase I study. Transplantation 2003;76:1079-84.
78. Brennan DC, Flavin 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.
79. Ortho Multicenter Transplant Study Group. A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. N Engl J Med 1985;313:337-42.
80. Norman DJ, Kahana L, Stuart FP Jr, et al. A randomized clinical trial of induction therapy with OKT3 in kidney transplantation. Transplantation 1993;55:44-50.
81. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. N Engl J Med 1990;323:1723-8.
82. Norman DJ, Vincenti F, de Mattos AM, et al. Phase I trial of HuM291, a humanized anti-CD3 antibody, in patients receiving renal allografts from living donors. Transplantation 2000;70:1707-12.
83. Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med 2002;346:1692-8.
84. Calne R, Friend P, Moffatt S, et al. Prope tolerance, perioperative campath 1H, and low-dose cyclosporin monotherapy in renal allograft recipients. Lancet 1998;351:1701-2. [Erratum, Lancet 1998;352:408.]
85. Kirk AD, Hale DA, Mannon RB, et al. Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (CAMPATH-1H). Transplantation 2003;76: 120-9.
86. Knechtle SJ, Pirsch JD, Fechner J Jr, et al. Campath-1H induction plus rapamycin monotherapy for renal transplantation: results of a pilot study. Am J Transplant 2003; 3:722-30.
87. Coles AJ, Wing M, Smith S, et al. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet 1999;354:1691-5.
88. Becker YT, Becker BN, Pirsch JD, Sollinger HW. Rituximab as treatment for refractory kidney transplant rejection. Am J Transplant 2004;4:996-1001.
89. Vincenti F, Kirkman R, Light S, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. N Engl J Med 1998;338:161-5.
90. Nashan B, Light S, Hardie IR, Lin A, Johnson JR. Reduction of acute renal allograft rejection by daclizumab. Transplantation 1999;67:110-5.
91. 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.]
92. 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.
93. Vincenti F. What's in the pipeline? New immunosuppressive drugs in transplantation. Am J Transplant 2002;2:898-903.
94. Hricik DE. Steroid-free immunosuppression in kidney transplantation: an editorial review. Am J Transplant 2002;2:19-24.
95. Cole E, Landsberg D, Russell D, et al. A pilot study of steroid-free immunosuppression in the prevention of acute rejection in renal allograft recipients. Transplantation 2001;72:845-50.
96. Kreis H, Cisterne JM, Land W, et al. Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. Transplantation 2000;69:1252-60.
97. Flechner SM, Goldfarb D, Modlin C, et al. Kidney transplantation without calcineurin inhibitor drugs: a prospective, randomized trial of sirolimus versus cyclosporine. Transplantation 2002;74:1070-6.
98. Smak Gregoor PJ, de Sevaux RG, Ligtenberg G, et al. Withdrawal of cyclosporine or prednisone six months after kidney transplantation in patients on triple drug therapy: a randomized, prospective, multicenter study. J Am Soc Nephrol 2002;13:1365-73.
99. Starzl TE, Murase N, Abu-Elmagd K, et al. Tolerogenic immunosuppression for organ transplantation. Lancet 2003;361:1502-10.
100. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000;342:605-12.
101. Port FK. Organ donation and transplantation trends in the United States, 2001. Am J Transplant 2003;3:Suppl 4:7-12.
102. Hariharan S, McBride MA, Cherikh WS, Tolleris CB, Bresnahan BA, Johnson CP. Post-transplant renal function in the first year predicts long-term kidney transplant survival. Kidney Int 2002;62:311-8.
103. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant 2004;4:378-83.
104. U.S. Renal Data System. USRDS 2003 annual data report: atlas of end-stage renal disease in the United States. Bethesda, Md.: National Institutes of Health, National Institutes of Diabetes & Digestive Kidney Diseases, 2003.
105. Sharples LD, McNeil K, Stewart S, Wall-

work J. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. J Heart Lung Transplant 2002;21:271-81.
106. Stewart KC, Patterson GA. Current trends in lung transplantation. Am J Transplant 2001;1:204-10.
107. Ojo AO, Held PJ, Port FK, et al. Chronic renal failure after transplantation of a nonrenal organ. N Engl J Med 2003;349:931-40.
108. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. Am J Transplant 2003;3:178-85.
109. Kasiske B, Cosio FG, Beto J, et al. Clinical practice guidelines for managing dyslipidemias in kidney transplant patients: a report from the Managing Dyslipidemias in Chronic Kidney Disease Work Group of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative. Am J Transplant 2004;4:Suppl 7:13-53.
110. Vanrenterghem Y, Ponticelli C, Morales JM, et al. Prevalence and management of anemia in renal transplant recipients: a European survey. Am J Transplant 2003;3:835-45.
111. Kasiske BL, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. Am J Transplant 2004;4:905-13.
112. Dharnidharka VR, Stablein DM, Harmon WE. Post-transplant infections now exceed acute rejection as cause for hospitalization: a report of the NAPRTCS. Am J Transplant 2004;4:384-9.
113. Matas AJ. What's new and what's hot in transplantation: clinical science ATC 2003. Am J Transplant 2003;3:1465-73.
114. Fritsche L, Einecke G, Fleiner F, Dragun D, Neumayer HH, Budde K. Reports of large immunosuppression trials in kidney transplantation: room for improvement. Am J Transplant 2004;4:738-43.
115. Hariharan S, McBride MA, Cohen EP. Evolution of endpoints for renal transplant outcome. Am J Transplant 2003;3:933-41.
116. Halloran PF. Call for revolution: a new approach to describing allograft deterioration. Am J Transplant 2002;2:195-200.
117. Warren DS, Zachary AA, Sonnenday CJ, et al. Successful renal transplantation across simultaneous ABO incompatible and positive crossmatch barriers. Am J Transplant 2004;4:561-8.
118. Fredericks S, Holt DW, MacPhee IA. The pharmacogenetics of immunosuppression for organ transplantation: a route to individualization of drug administration. Am J Pharmacogenomics 2003;3:291-301.
119. Cattaneo D, Perico N, Remuzzi G. From pharmacokinetics to pharmacogenomics: a new approach to tailor immunosuppressive therapy. Am J Transplant 2004;4:299-310.
120. MacPhee IA, Fredericks S, Tai T, et al. Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and P-glycoprotein correlate with dose requirement. Transplantation 2002;74:1486-9.
121. *Idem*. The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. Am J Transplant 2004;4:914-9.
122. Auchincloss H Jr. In search of the elusive holy grail: the mechanisms and prospects for achieving clinical transplantation tolerance. Am J Transplant 2001;1:6-12.
123. Schwartz R, Dameshek W. Drug-induced immunological tolerance. Nature 1959;183:1682-3.
124. Matthews JB, Ramos E, Bluestone JA. Clinical trials of transplant tolerance: slow but steady progress. Am J Transplant 2003;3:794-803.