Immunosuppressive Drug Therapy

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The first successful kidney transplantation between monozygotic identical twins did not require any immunosuppressive drugs. Clinical application of azathioprine and glucocorticosteroids allowed the transfer of organs between genetically disparate donors and recipients. Transplantation is now the standard of care, a life-saving procedure for patients with failed organs. Progress in our understanding of the immunobiology of rejection has been translated to the development of immunosuppressive agents targeting T cells, B cells, plasma cells, costimulatory signals, complement products, and antidonor antibodies. Modern immunopharmacologic interventions have contributed to the clinical success observed following transplantation but challenges remain in personalizing immunosuppressive therapy.

Organ transplantation, in the absence of immunosuppressive drugs, can be performed between monozygotic identical twins, and the risk of immune rejection is invariant in the absence of drug therapy in almost all donor–recipient combinations. The goal of immunosuppressive therapy is to prevent the graft destructive immune response. Whether rejection prophylaxis strategies prevent the development of a tolerogenic response remains unresolved.

In the decades leading up to 1980, azathioprine and glucocorticosteroids were the primary immunosuppressive drugs. The introduction of calcineurin inhibitors (CNI), cyclosporine (CsA), and tacrolimus (Tac) in the 1980s ushered in an era of improved graft outcome. Small molecules and biologics became available as a result of advancements in drug design and use of recombinant DNA technology. Consequently, transplant clinicians/patients now have an

array of agents such as mycophenolate mofetil (MMF), sirolimus, rabbit-antithymocyte globulin (rATG), alemtuzumab, and belatacept for clinical use. Treatment for steroid-resistant rejection is now feasible with novel agents such as rATG. Agents with direct efficacy against the humoral antiallograft response appeared to have improved the outcomes of patients with antibody-mediated rejection. However, we lack long-term data regarding efficacy and toxicity of the newer drugs. Moreover, adverse events such as polyomavirus infection and posttransplant EBV-associated lymphoma are directly related to the increased potency of newer agents. Importantly, the improvement in short-term outcome following their introduction has not extended substantively the life span of transplanted organs. Immunosuppressive agents are also increasingly used in novel protocols to induce transplant tolerance.

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We briefly review the immunobiology of the antiallograft response to provide the conceptual framework for the clinical application of multidrug regimens to constrain the antiallograft repertory.

IMMUNOBIOLOGY OF REJECTION

Allograft rejection involves a highly orchestrated action of multiple cell types and mediators. Effective immunosuppression is achieved by targeting these cells and mediators at multiple

levels (Fig. 1). Lymphocytes are the principal immune cells for the identification of the foreignness of the allograft and mediate graft damage (rejection) by cell-to-cell interactions and via their secretory products including antibodies that bind to antigens displayed by the allograft and recruit complement components (complement-dependent cytotoxicity) and/or Fc receptor-bearing cells (antibody-dependent cell-mediated cytotoxicity).

The α and β chains on the T cell that recognizes the peptide-major histocompatibility

Figure 1. The antiallograft response and sites of action of common immunosuppressive drugs. Schematic representation of human leukocyte antigen (HLA), the primary stimulus for the initiation of the antiallograft response; cell surface proteins participating in antigenic recognition and signal transduction; contribution of the cytokines and multiple cell types to the immune response; and the potential sites for the action of commonly used immunosuppressive drugs. Figure 2 shows the cell surface proteins on antigen-presenting cells (APCs) interacting with T cells to generate costimulatory/coinhibitory signals. (Adapted from Suthanthiran and Strom 1994; reprinted, with permission, from the authors.)

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Figure 2. T-cell/APC contact sites and potential targets of immunosuppressive drugs. Schematic representation of T-lymphocyte and antigen-presenting cell contact sites. Signal transduction in T cells on recognition of antigen is not by the TCR itself, but proteins CD3 and ζ noncovalently linked to the TCR. Signaling of T cells via the TCR/CD3 complex (antigenic signal) is necessary, but insufficient in itself to induce maximal T-cell proliferation; plenary activation is dependent on both the antigenic signals and the costimulatory signals engendered by the physical interactions among the cell-surface proteins expressed on antigen-specific T cells and those displayed on APCs. Of all the APCs, mature dendritic cells express the highest level of costimulatory proteins. The best-characterized T-cell costimulation pathway is the interaction of CD28 protein on the T-cell surface with the B7-1 and B7-2 (CD80 and CD86) proteins expressed on activated APCs. In the absence of this second signal, T cells either remain unresponsive or become actively tolerant to antigens. CD28-mediated signals increase the production of cytokines as well as promote the survival of T cells by increasing the expression of antiapoptotic proteins. Although costimulatory pathways were discovered as mediators of T-cell activation, homologous molecules are involved in inhibiting T-cell activation. The key inhibitory receptor is the CTLA-4, a member of CD28 family. The higher the affinity of CTLA-4, as compared to CD28, to B7 may determine the differential binding of stimulatory CD28 and inhibitory CTLA-4 to the same B7 on APCs. Several molecules on the T cells or APCs are potential targets for immunosuppressive drug development. Monoclonal antibodies (anti-CD25, anti-CD2) and recombinant fusion proteins (belatacept) targeting specific cell-surface contact sites are available or undergoing clinical trials as immunosuppressive or tolerance-inducing drugs. (Adapted from Suthanthiran 1996; reprinted, with permission, from the author.)

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complex on the surface of antigen-presenting cells (APCs) is the clonotypic T-cell receptor (TCR). Signal transduction in T cells on recognition of antigen is not by the TCR itself, but proteins CD3 and ζ chain noncovalently linked to the TCR. CD4 and CD8 proteins, coreceptors involved in T-cell activation, are expressed on reciprocal T-cell subsets and bind to nonpolymorphic domains of human leucocyte antigen (HLA) class II (DR, DP, DQ) and class I (A, B, C) molecules, respectively. Following activation by antigen, the TCR/CD3 complex and coclustered CD4 and CD8 activate protein tyrosine kinases that are associated with the cytoplasmic tail of CD4 or CD8 and result in activation of several downstream pathways (Brown et al. 1989; Suthanthiran 1990; Beyers et al. 1992; Lebedeva et al. 2004; Fooksman et al. 2010).

Antigenic signaling of T cells via the TCR/CD3 complex is necessary, but insufficient in itself to induce maximal T-cell proliferation; plenary activation is dependent on both the antigenic signals and the costimulatory signals engendered by the physical interactions among the cell-surface proteins expressed on antigen-specific T cells and those displayed on APCs (Fig. 2) (Suthanthiran and Garovoy 1983). Among multiple types of APCs, mature dendritic cells express the highest level of costimulatory proteins and are the most potent antigen-presenting cells. Although some of the costimulatory proteins are expressed in naïve T cells, several of them are expressed following activation of T cells. The best-characterized T-cell costimulation pathway is the interaction of CD28 protein on T cells with the B7-1 and B7-2 (CD80 and CD86) proteins on APCs. In the absence of this second signal, T cells either remain unresponsive or become tolerant to antigens. Although costimulatory pathways were discovered as mediators of T-cell activation, homologous molecules are involved in inhibiting T-cell activation. The key inhibitory receptor is the cytotoxic T-lymphocyte antigen 4 (CTLA-4), a member of CD28 family. The higher the affinity of CTLA-4, as compared to CD28, to B7 may determine the differential binding of stimulatory CD28 and inhibitory CTLA-4 to the same B7 on APCs.

Signal transduction by the TCR/CD3 complex culminates in the transcription of several genes, which includes T-cell growth, survival, and differentiation factor IL-2. Secreted IL-2 signals through IL-2 receptors are induced on activation. The IL-2 receptor signaling proceeds through multiple pathways: Shc/Ras/Raf-1/MAP kinase, JAK1/JAK3/STAT5, and PI 3-kinase/AKT/p70 S6 kinase pathway.

B cells recognize antigens and are activated in the lymphoid tissues. The B-cell antigen receptor complex is made of membrane IgM and IgD associated with the invariant Ig α and Ig β molecules. The Ig α and Ig β molecules in B cells function similar to CD3 and ζ proteins in the T cells. Complement components play an important role in B-cell activation. B cells express receptor for C3d, a degradation product of complement factor 3 (C3), called CR2 (CD21). The CD21-CD19-CD81 proteins on the B-cell membrane are termed the B-cell coreceptor complex. Antigen and C3d binding to the coreceptor complex activates several kinases resulting in B-cell activation (Batista and Harwood 2009). B-cell response to protein antigens requires recognition of the antigen by the T helper cells and antigen-specific T- and B-cell cooperation (Batista and Harwood 2009). Activated B cells differentiate into antibody-secreting plasma cells.

The net consequence of cytokine production and acquisition of cell-surface receptors for these transcellular molecules by the T cells is the emergence of antigen-specific and graft-destructive T cells. With help from T cells, the humoral arm of immunity is activated resulting in production of donor-specific antibodies. CD8⁺ cytotoxic T-lymphocyte (CTL)-mediated killing of target cells is mainly accomplished by the directed release of perforin and granzyme, as well as by FasL–Fas interaction, all of which lead to the activation of several apoptotic pathways. Antibodies cause target cell destruction by complement-dependent or complement-independent mechanisms.

IMMUNOSUPPRESSIVE AGENTS

Immunosuppressive medications can be classified into induction or maintenance agents

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Table 1. Induction agents

Drug	Mechanisms of action	FDA approved	Off label
rATG	Polyclonal antibodies against CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, HLA-DR, HLA I heavy chain	FDA approved as antirejection drug Off label as induction agent	
Basiliximab	Chimeric human/murine monoclonal IgG _{1κ} against CD25/Tac subunit	Yes	No
Alemtuzumab	Humanized monoclonal IgG _{1κ} against CD52	No	Yes
Muromonab-CD3 (OKT3)	Murine monoclonal antibody against CD3 of T-cell receptor complex	FDA approved as antirejection drug Off label as induction agent Voluntarily discontinued	

FDA, Food and Drug Administration.

(Tables 1 and 2). Induction agents serve to eliminate alloreactive lymphocytes when the host's immune system is first exposed to alloantigens following transplantation. Several of the biologics such as rATG and OKT3 were approved for use as antirejection therapy rather than as induction agents. Maintenance agents provide continuous prophylaxis against rejection. Approved and off-label use of specialty drugs exert their

effects against B cells and plasma cells or directly inhibit downstream complement activation following antibody binding (Table 3).

Induction Agents***Antithymocyte Globulin (ATG)***

Antithymocyte globulin (ATG) is produced by immunizing rabbits or horses with human thy-

Table 2. Maintenance agents

Drugs	Mechanisms	FDA approved	Off label
Azathioprine	Converted to 6-mercaptopurine, inhibits purine biosynthesis and CD28 signaling	Yes	No
Glucocorticoids	Inhibits formation of free NF-κB and down-regulates expression of proinflammatory cytokines	NA	Yes
Cyclosporine	Binds cyclophilin and inhibits calcineurin, prevents activation of NFAT and expression of IL-2, stimulates TGF-β expression	Yes	No
Tacrolimus	Binds FK-binding protein, inhibits calcineurin, prevents activation of NFAT and expression of IL-2, stimulates TGF-β expression	Yes	No
Mycophenolate mofetil	Inhibits inosine monophosphate dehydrogenase and de novo purine biosynthetic pathway	Yes	No
Mycophenolic acid	Enteric coated, inhibits inosine monophosphate dehydrogenase and de novo purine biosynthetic pathway	Yes	No
Sirolimus	Binds FK-binding protein and inhibits mammalian target of rapamycin (mTOR) pathway by binding mTOR complex 1 leading to blockade of activation of 70-kDa S6 protein kinases, expression of bcl-2 proto-oncogene, Ca ²⁺ -independent CD28-induced costimulatory pathway	Yes	No
Everolimus	Derivative of rapamycin	Yes	No
Belatacept	Fusion protein of modified CTLA-4-human Ig, blocks B7/CD28 costimulatory proteins	Yes	No
Leflunomide	Inhibits dihydroorotate dehydrogenase and pyrimidine biosynthesis	No	Yes

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Table 3. Agents with activities against B cell, plasma cell, and complement components

Drug	Mechanisms	FDA approved	Off label
Bortezomib	Proteasome inhibitor	No	Yes
Eculizumab	Monoclonal antibody against complement C5	No	Yes
Rituximab	Monoclonal antibody against CD20 glycoprotein on B cells	No	Yes
IVIG	Suppress autoantibodies, cytokines, neutralize complements, up-regulate FcγRIIB, immunomodulation	No	Yes

IVIG, intravenous immune globulins.

mocytes generating polyclonal antithymocyte antibodies. The major mode of action for ATG is the depletion of T cells. ATG also targets a wide range of antigens displayed on B cells, dendritic cells, NK cells, and endothelial cells, and disrupts cell trafficking (Mueller 2007). The equine ATG (eATG) product ATGAM became available in the 1980s, whereas rabbit-ATG (rATG) or thymoglobulin was introduced commercially in 1999. rATG or eATG are used both as induction agents, especially in high-risk renal transplant recipients, and for the treatment of moderate to severe acute rejection. Head-to-head comparison study of rabbit versus equine ATG preparations in a randomized double-blinded fashion showed that the acute rejection rate was lower with rATG compared to eATG (4% vs. 25%, RR = 0.09, $P = 0.009$) (Brennan et al. 1999). At 10 yr, the acute rejection rate was 11% for rATG arm and 42% for eATG arm ($P = 0.004$) (Hardinger et al. 2008). Currently, rATG is the most common induction agent in kidney graft recipients.

Interleukin (IL)-2 Receptor Antagonists: Basiliximab and Daclizumab

Both basiliximab and daclizumab are monoclonal antibodies (mAbs) directed at the IL-2 receptor α (CD25 antigen); basiliximab is a chimeric mAb, whereas daclizumab is a humanized mAb. Both antibodies received Food and Drug Administration (FDA) approval as an induction agent for renal transplantation. A meta-analysis showed that when combined with a standard double or triple immunosuppressive regimen, these antibodies reduced the incidence of acute rejection by 34% and the incidence of steroid-resistant rejection by 49%, and that their efficacy

in preventing acute rejection was similar to that of OKT3 and polyclonal antibody preparations and, importantly, with fewer side effects (Webster et al. 2004). A prospective randomized international trial tested the efficacy of a 5-day course of ATG versus two doses of basiliximab therapy in 278 deceased-donor renal transplants at risk for acute rejection or delayed graft function (DGF) (Brennan et al. 2006). Participants in the ATG group ($n = 141$) had a lower incidence of acute rejection (15.6% vs. 25.5%) at 12 mo compared to the basiliximab group ($n = 137$). The incidence of DGF, death, and graft loss was similar in the two groups. A Cochrane systematic review of 71 studies (10,537 participants) compared the effects of IL-2Ra to placebo in kidney graft recipients found reduced graft loss because of death with a functioning graft by 25% at 1 yr (RR 0.75, 95% CI, 0.62–0.90) (Webster et al. 2010). The incidence of biopsy-proven acute rejection at 1 yr was also reduced by 28% (RR 0.72, 95% CI, 0.64–0.81). Compared to ATG, induction with IL-2Ra resulted in similar rate of graft loss at any time point and an equivalent incidence of clinical rejection. However, ATG was superior to IL-2Ra when comparing biopsy-proven acute rejection at 1 yr (RR 1.30, 95% CI, 1.01–1.67), but at a cost of 75% increase in malignancy (RR 0.25, 95% CI, 0.07–0.87) and 32% increase in cytomegalovirus (CMV) disease (RR 0.68, 95% CI, 0.50–0.93).

Alemtuzumab

Alemtuzumab (Campath-1H), a humanized monoclonal IgG_{1k} directed against the CD52 glycoprotein and developed by the pathology department at Cambridge University, was approved by the FDA for B-cell chronic lympho-

cytic leukemia. The INTAC study group tested alemtuzumab against conventional induction agents in a randomized prospective multicenter trial of kidney graft recipients (Hanaway et al. 2011). All participants received Tac and MMF maintenance immunosuppression with rapid steroid discontinuation after 5 days of therapy. Compared to basiliximab, alemtuzumab induction in the low-risk group resulted in a lower rate of biopsy-proven acute rejection at 6 mo (2% vs. 18%, $P < 0.001$), 12 mo (3% vs. 20%, $P < 0.001$), and 36 mo (10% vs. 22%, $P = 0.003$). Compared to rATG, alemtuzumab induction in the high-risk group resulted in equivalent rates of biopsy-proven acute rejection at 6 mo (6% vs. 9% $P = 0.49$), 12 mo (10% vs. 13%, $P = 0.53$), and 36 mo (18% vs. 15%, $P = 0.63$). Patient survival at 3 yr was similar between alemtuzumab and basiliximab in the low-risk group (95% vs. 98%, $P = 0.19$) or between alemtuzumab and ATG in the high-risk group (99% vs. 91%, $P = 0.07$). The rates of late biopsy-proven acute rejection (between 12 and 36 mo) were higher in the alemtuzumab cohort when compared to participants in the conventional induction cohorts (8% vs. 3%, $P = 0.03$). After censoring for deaths, graft survival at 3 yr was also similar in the low-risk (97% vs. 94%, $P = 0.17$) or the high-risk group (91% vs. 84%, $P = 0.32$).

Muromonab—CD3 (OKT3)

OKT3, a mAb directed against the CD3 epsilon component of the CD3 complex and the first of its class to be applied in the clinic, received FDA approval as an antirejection treatment in 1986. A randomized, controlled trial showed that OKT3 reversed 94% of acute rejection episodes in 63 kidney graft recipients compared to 75% in the 60 recipients treated with corticosteroids ($P = 0.009$) (Ortho Multicenter Transplant Study Group 1985). Before its voluntary withdrawal from the U.S. market, OKT3 was used as an induction agent in renal transplantation and for the treatment of steroid-resistant acute rejection. OKT3-associated first-dose reaction as a result of cytokine release may be severe and includes fever, chills, respiratory symptoms, and head-

aches. A humanized preparation (HuM291) with lesser cytokine release reaction was investigated in a phase I study of kidney graft recipients (Norman et al. 2000) and in patients with new onset insulin-dependent diabetes mellitus (Herold et al. 2002).

Maintenance Drugs for Prophylaxis against Rejection

Azathioprine

Azathioprine (AZA) is a prodrug of 6-mercaptopurine and inhibits purine biosynthesis, and blocks CD28 costimulatory signaling via Rac 1 (Tiede et al. 2003). In a randomized conversion trial from MMF to AZA in 48 stable kidney transplant recipients at 6 mo following transplantation, acute rejection rates were comparable (4.5% vs. 3.8%) after a 6-mo observation period in the MMF ($n = 22$) or AZA ($n = 26$) arm. High-risk patients were, however, excluded in this trial (Wuthrich et al. 2000).

Glucocorticosteroids

Glucocorticosteroids serve as first-line agents for treating rejection. The mechanistic basis for their anti-inflammatory properties is diverse (Rhen and Cidlowski 2005). On binding the glucocorticoids receptor, the steroid-receptor complex inhibits the expression of proinflammatory genes/proteins dependent on NF- κ B and activator protein 1. Glucocorticosteroid and its receptor complex may also block the transcription of IL-2 via disruption of key DNA-binding proteins (Vacca et al. 1992). High-dose glucocorticosteroids are given peri-transplantation and tapered to a lower dose for the life of the allograft. Serious side effects because of prolonged steroid exposure supported the implementation of a steroid-sparing regimen for kidney transplantation. At our center, following rATG induction, the cumulative incidence of acute kidney rejection with an early corticosteroid withdrawal regimen in kidney graft recipients was 12.0% over a 5-yr period and death-censored graft survival was 96.2%, 91.9%, and 87.6% at 1, 3, and 5 yr, respectively (Aull et al. 2012).

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A Cochrane systematic review of 5949 participants in 30 randomized controlled trials showed that glucocorticosteroid-sparing regimens had no effect on patient mortality or graft loss including death with functioning graft (Pascual et al. 2009). However, patients on glucocorticosteroid-sparing regimens were at a higher risk of graft loss (excluding death) when compared to patients on conventional glucocorticosteroid regimen (RR 1.23, 95% CI, 1.00–1.52). The risk was higher in patients not concurrently on MMF/mycophenolic acid or everolimus (RR 1.70, 95% CI, 1.00–2.90). Compared with standard glucocorticosteroid-containing regimen and in the presence of CsA, the risk of acute rejection was higher without glucocorticosteroid maintenance therapy (RR 1.27, 95% CI, 1.14–1.40). Maintenance immunosuppression without glucocorticosteroid was beneficial in reducing antihypertensive drug need, cholesterol-lowering drug need, new-onset diabetes after transplantation, cardiovascular events, and the incidence of infection.

Calcineurin Inhibitors: Cyclosporine (CsA) and Tacrolimus (Tace)

CsA is a cyclic nonribosomal peptide of 11 amino acids isolated from the soil fungus *Tolypocladium inflatum*. CsA binds cyclophilin, an evolutionarily conserved peptidyl prolyl *cis-trans* isomerase, and the CsA and cyclophilin heterodimer targets and inactivates calcineurin, a serine-threonine phosphatase. Inhibition of calcineurin activity is central to the immunosuppressive effects of both CsA and Tac and results in lack of dephosphorylation of the nuclear factor of activated T-cells protein-1 (NFAT-1), nuclear import, and inhibition of NFAT-dependent transcription of a number of cytokine genes including IL-2. CsA as well as Tac enhances the expression of transforming growth factor β (TGF- β) (Sehajpal et al. 1993; Khanna et al. 1999). Because TGF- β is a potent inhibitor of T-cell proliferation and generation of antigen-specific CTL (Kehrl et al. 1986), heightened expression of TGF- β must contribute to the antiproliferative/immunosuppressive activity of CsA/Tac. This TGF- β -inducing effect of

CsA/Tac suggests also a mechanism for renal fibrosis and tumor metastasis associated with CNIs because TGF- β is a fibrogenic and pro-angiogenic cytokine.

A Cochrane systematic review comparing Tac versus CsA regimen in 4102 participants from 30 studies (Webster et al. 2005) showed that Tac regimen reduced graft loss for up to 3 yr (RR 0.56, 95% CI, 0.36–0.86). Metaregression showed that this benefit was less at higher target trough levels of Tac ($P = 0.04$), after allowing for differences in CsA formulation ($P = 0.97$) and target trough level ($P = 0.38$). At 1 yr, Tac regimen resulted in less acute rejection (RR 0.69, 95% CI, 0.60–0.79) or steroid-resistant rejection (RR 0.49, 95% CI, 0.37–0.64). Tac-treated patients had more insulin-dependent diabetes mellitus (RR 1.86, 95% CI, 1.11–3.09), tremor, headache, and gastrointestinal symptoms such as diarrhea, dyspepsia, and vomiting. CsA-treated patients had more constipation and cosmetic side effects. The review revealed no significant differences in infection or malignancy between CsA and Tac regimens.

MMF and Enteric-Coated Mycophenolate Sodium (EC-MPS)

MMF is the prodrug of mycophenolic acid and reversibly inhibits inosine monophosphate dehydrogenase, an enzyme in the *de novo* pathway of guanosine biosynthesis. B and T lymphocytes are dependent on this biosynthetic pathway to satisfy their guanosine requirements. The FDA approved MMF for the prevention of kidney transplant rejection in 1995. Early clinical trials used MMF to replace azathioprine in the cyclosporine- and glucocorticosteroid-based immunosuppressive regimen. These controlled, prospective trials showed a diminished incidence of early acute rejection (Sollinger 1995; Lui and Halloran 1996; European Mycophenolate Mofetil Cooperative Study Group 1999). Although follow-up studies over a 3-yr period have indicated an advantage for MMF over azathioprine (European Mycophenolate Mofetil Cooperative Study Group 1999), a randomized trial comparing MMF with azathioprine in recipients

of a first kidney transplant from a deceased donor found similar levels of acute rejection in the first 6 mo of transplantation (Remuzzi et al. 2004).

Enteric-coated mycophenolate sodium (EC-MPS) was developed to improve the gastrointestinal tolerability of MPA. An international phase III, randomized, double-blind, parallel group trial showed the therapeutic equivalence of MMF and EC-MPS (Salvadori et al. 2004). At 12 mo, the incidence of acute rejection, graft loss, and death was comparable for both groups and gastrointestinal complications were not different.

Sirolimus

Sirolimus was discovered in 1975 on the island of Rapa Nui (Easter Island) from *Streptomyces hygroscopicus* in the soil sample. Also known as rapamycin, it is a macrocyclic lactone that, like Tac, binds FKBP. However, sirolimus and Tac affect different and distinctive sites downstream in the signal transduction pathway. Whereas sirolimus blocks IL-2 and other growth factor-mediated signal transduction, Tac does not. In addition, the sirolimus–FKBP complex, unlike the Tac–FKBP complex, does not bind calcineurin. The antiproliferative activity of the sirolimus–FKBP complex is linked to the blockade of the activation of 70-kDa S6 protein kinases and blockade of expression of the bcl-2 proto-oncogene. Sirolimus also blocks the Ca^{2+} -independent CD28-induced costimulatory pathway. Sirolimus was approved in 1999 by the FDA and indicated for the prophylaxis against kidney rejection.

The Cochrane group analyzed the use of mTOR inhibitor for kidney transplant immunosuppression in 33 studies (7114 participants) including 27 sirolimus, five everolimus, and one head-to-head trial (Webster et al. 2006). Replacing CNI with mTOR inhibitor resulted in no difference in acute rejection, but lower serum creatinine (mean difference $-18.31 \mu\text{M/L}$, -30.96 to -5.67), and higher risk of bone marrow suppression (leukopenia: RR 2.02, 95% CI, 1.12–3.66; thrombocytopenia: RR 6.97, 95% CI, 2.97–16.36, and anemia: RR 1.67, 95% CI,

1.27–2.20). Replacing antimetabolites with mTOR inhibitor resulted in lower risk of acute rejection (RR 0.84, 95% CI, 0.71–0.99) and CMV infection (RR 0.49, 95% CI, 0.37–0.65), but higher risk of dyslipidemia (RR 1.65, 95% CI, 1.32–2.06).

Everolimus (RAD)

Everolimus is a derivative of sirolimus. The FDA approved everolimus in 2010 for prevention of kidney transplant rejection following its approval in 2009 for the treatment of advanced renal cell carcinoma in patients who have failed sunitinib or sorafenib therapy. The use of everolimus in phase II clinical trials involving cyclosporine, steroids, and basiliximab induction resulted in excellent graft survival at 36 mo (Nashan et al. 2004). In a short-term phase III trial, everolimus was comparable to MMF with cyclosporine and steroids in preventing acute rejection (Vitko et al. 2004).

Belatacept

Belatacept is a recombinant protein created by fusion of modified CTLA-4 with an Fc portion of human immunoglobulin (CTLA-4Ig) and was designed to block the B7/CD28 costimulatory pathway. The FDA approved belatacept in 2011 for the prophylaxis of rejection in kidney transplant recipients. Phase III trials, BENEFIT, and BENEFIT-EXT have tested the effectiveness of belatacept as part of a CNI-free regimen (Durrbach et al. 2010; Vincenti et al. 2010). The BENEFIT study was a 3-yr randomized, parallel group designed trial conducted at 100 transplant sites worldwide. Following basiliximab induction, participants were randomized to one of three groups consisting of a more intensive belatacept (MI) regimen, a less intensive belatacept regimen (LI), or cyclosporine with the addition of maintenance MMF and corticosteroids. The incidence of acute rejection at 12 mo was higher in belatacept groups (22% and 17%) compared to cyclosporine groups (7%). More participants also developed type IIa and IIb rejections in belatacept groups, but without an increase in donor-specific antibody production compared to

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cyclosporine-treated groups. The mean glomerular filtration rate (GFR) was better at 12 mo for belatacept groups compared to cyclosporine groups.

The BENEFIT-EXT trial was a 3-yr randomized, multicenter trial performed at 79 transplant sites worldwide to test the benefit of a CNI-free regimen containing belatacept in patients undergoing high risk transplant from expanded criteria donors. Following basiliximab induction, participants were randomized to one of three groups (MI, LI, cyclosporine) with the addition of maintenance MMF and corticosteroids. The incidence of acute rejection was not different among the three groups, but CNI-free belatacept regimens resulted in more type IIb rejections. The mean GFR was higher at 12 mo for MI groups (52.1 mL/min/1.73 m²), but not for LI groups (49.5 mL/min/1.73 m²) compared to cyclosporine groups (45.2 mL/min/1.73 m²). Both the BENEFIT and BENEFIT-EXT trials showed that neither the MI nor LI belatacept regimen was noninferior to cyclosporine on patient and graft survival. At the 3-yr follow-up for the BENEFIT trial, the mean calculated GFR (cGFR) decreased by 2.0 mL/min/1.73 m² per year for cyclosporine groups, whereas mean cGFR increased in MI and LI groups (+1.0 mL/min/1.73 m² and +1.2 mL/min/1.73 m² per year, respectively) (Vincenti et al. 2012). The 3-yr follow-up study of BENEFIT-EXT showed similar patient survival in the three groups (MI 80%, LI 82%, and 80% in cyclosporine groups) (Pestana et al. 2012). The mean cGFR was 11 mL/min higher in belatacept-treated arms when compared to cyclosporine arms (MI 42.7 mL/min, LI 42.2 mL/min, cyclosporine 31.5 mL/min). Patients in the cyclosporine group were more likely to progress to a GFR of less than 30 mL/min when compared to MI and LI groups (44% vs. 27% and 30%, respectively).

Conversion from a CNI-based to a belatacept-based immunosuppression regimen was investigated in a randomized phase II study (Rostaing et al. 2011). Patients who were more than 6 mo but less than 3 yr after transplant were randomized to stay on CNI ($n = 89$) or undergo conversion to belatacept ($n = 84$). Post hoc

analysis showed that conversion to belatacept resulted in improvement of mean cGFR at 1 yr when compared to baseline (7.0 mL/min belatacept vs. 2.1 mL/min for CNI group; $p = 0.0058$). Acute rejection occurred within 6 mo after conversion to a CNI-free regimen in six patients.

Leflunomide

Leflunomide is a disease-modifying antirheumatic drug approved by the FDA in 1998 for treatment of rheumatoid arthritis. It belongs to the family of drugs known as malonitrilamides and is a synthetic isoxazole derivative that inhibits dihydroorotate dehydrogenase—a key enzyme for de novo pyrimidine synthesis. Leflunomide has antiviral effects against CMV, herpes simplex virus type 1, and polyomavirus (Waldman et al. 1999; Knight et al. 2001; Farasati et al. 2005). A short-term, open-label, prospective crossover trial of leflunomide of 22 patients with chronic renal allograft dysfunction found 100% patient survival and 91% graft survival at 6-mo posttransplantation, and was well tolerated, with anemia being the most common adverse effect (Hardinger et al. 2002).

A systematic review of the treatment of polyomavirus infection in kidney transplant patients showed that the pooled death-censored graft loss rate was 8/100 patient-yr for reduction of immunosuppression and 13/100 patient-yr for the addition of leflunomide (Johnston et al. 2010). The utility of leflunomide for polyomavirus-associated nephropathy remains undefined.

Agents against B Cells, Plasma Cells, Complements

For agents discussed in this section, none are FDA approved for transplantation.

IVIG

Immune globulin therapy has been available since the 1950s for treatment of primary immunodeficiency diseases. The intravenous

preparation is better tolerated and used to treat autoimmune diseases based on its immunomodulatory effects (Gelfand 2012). In renal transplantation, IVIG is utilized to reduce humoral immunity in two settings: (1) reduce the level of preexisting anti-HLA antibodies and convert a positive cross-match recipient to a negative cross-match recipient (Jordan et al. 2004), and (2) treat antibody-mediated rejection (White et al. 2004).

A combination of IVIG and rituximab has facilitated, in a safe manner, rapid transplantation of 16 of 20 highly sensitized patients (Vo et al. 2008). A protocol incorporating plasmapheresis and low-dose IVIG was successful in desensitizing 211 HLA-sensitized patients before kidney transplant (Montgomery et al. 2011). The study showed that compared with a matched control group of wait-listed patients who remained on dialysis, the patient survival rate of desensitized patients following kidney transplant was far superior after 8 yr of follow-up (30.5% vs. 80.6%, respectively; $P < 0.001$).

A desensitization regimen of IVIG (2 g/kg, maximum dose 120 g) and rituximab (375 mg/m²) was tested on highly sensitized kidney transplant candidates with calculated panel reactive antibody (cPRA) of greater than 50% (Marfo et al. 2012). Two of 11 patients who completed the protocol received a kidney transplant compared to 14 of 27 patients in the non-desensitized cohort. Desensitization using one dose of rituximab and high-dose IVIG was not successful in reducing class I and class II cPRA levels.

Rituximab

Rituximab is a chimeric murine/human monoclonal IgG_{1κ} directed against the CD20 antigen expressed on B cells. Rituximab is FDA approved for the treatment of CD20-positive, B-cell non-Hodgkin's lymphoma. Initial experience with rituximab shows promise in the treatment of steroid-resistant acute renal allograft rejection (Becker et al. 2004). Rituximab has been used as a component of a preconditioning regimen to prepare patients for renal transplan-

tation from ABO incompatible donors (Tyden et al. 2005). Rituximab may exert a direct effect on podocyte actin cytoskeleton by interacting with sphingomyelin phosphodiesterase acid-like 3b protein (Fornoni et al. 2011). Pilot studies have yielded mixed outcomes when rituximab is added to a regimen of plasmapheresis to induce remission in recurrent focal segmental glomerulosclerosis (Yabu et al. 2008; Dello Strologo et al. 2009).

Bortezomib

Bortezomib is the first proteasome inhibitor approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. Proteasomes are large cytosolic protease complexes and with ubiquitin they perform basic house-keeping protein degradation in all eukaryotic cells. The ubiquitin-proteasome pathway is essential for physiologic functions such as oncogenesis, inflammation, apoptosis, cell cycle progression, and immune activation. Plasma cells are professional antibody-secreting cells, and in the process of producing antibodies they are subjected to tremendous intracellular stress leading to proteasomal insufficiency and cell death if accumulation of polyubiquitinated proteins is left unchecked (Perry et al. 2009). Studies in kidney transplant recipients suggest that nonmalignant plasma cells are susceptible to proteasome inhibition (Perry et al. 2009). Preliminary results of bortezomib in antibody-mediated kidney rejection are promising (Everly et al. 2008).

Eculizumab

Eculizumab is a humanized monoclonal antibody against complement 5a molecule and approved by the FDA for the treatment of paroxysmal nocturnal hematuria. Several case reports described the use of eculizumab in renal transplant recipients with atypical hemolytic-uremic syndrome, as salvage therapy for antibody-mediated rejection, and renal transplant patients with catastrophic antiphospholipid antibody syndrome (Locke et al. 2009; Lonze et al. 2010; Zimmerhackl et al. 2010).

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Novel Drugs in the Pipeline

Alefacept

Alefacept is a dimeric fusion protein made by linking the CD2-binding portion of the human lymphocyte function-associated antigen-3 to the Fc portion of human IgG1. It was marketed as Amevive and indicated for the treatment of psoriasis. A phase 2 randomized open-label parallel group multicenter trial enrolled 309 subjects and randomized kidney graft recipients to four arms: control (basiliximab induction with full-dose Tac, MMF, and steroids), alefacept/low-dose Tac/MMF/steroids (A), alefacept/full-dose Tac/steroids (B), and every other week alefacept/low-dose Tac/MMF/steroids (C) (Bromberg et al. 2011). The incidence of BPAR was higher in group A compared to the control arm (26.3% vs. 12.7%, $P < 0.05$) whereas groups B and C had similar rates of biopsy-proven acute rejection compared to the controls (18.8% and 16.7%, respectively). At 6 mo, patient and graft survival as well as renal function were similar in all groups.

Sipilizumab (MEDI-507)

Sipilizumab is a humanized monoclonal antibody directed against CD2 antigen and has not received FDA approval. It has been investigated as an induction agent in a pioneering study of kidney transplant tolerance following bone marrow-induced mixed chimerism, and is the only monoclonal antibody shown to date to induce tolerance to MHC-incompatible kidney allografts in humans (Kawai et al. 2008).

Sotrastaurin (AEB-071)

AEB-071, a selective protein kinase C isoforms inhibitor, prevents T-cell activation independent of calcineurin inhibition and is being developed for the prophylaxis of kidney transplant rejection. Phase II studies in de novo kidney transplant recipients randomized to sotrastaurin ($n = 81$) showed a higher composite efficacy failure rate at 3 mo without CNi (Friman et al. 2011). Biopsy-proven acute rejection occurred in 17 patients in the sotrastaurin arm.

The median eGFR at 3 mo was higher in patients who have not received Tac (59.0 ± 22.3 vs. 49.5 ± 17.7 mL/min/1.72 m², $P = 0.006$).

Janus Kinase (JAK)3 Inhibitor (CP-690550)

Tofacitinib inhibits the tyrosine kinase required for signal transduction downstream of cytokine receptors and is important for the activation and function of T cells as well as natural killer cells. It was approved in 2012 by the FDA for the treatment of rheumatoid arthritis. A phase IIA randomized, open-label multicenter trial was conducted in de novo kidney transplant recipients (Busque et al. 2009). Following induction with IL-2Ra mAbs, participants were randomized to lower-dose JAK3 inhibitor (CP15), higher-dose JAK3 inhibitor (CP30), or Tac and with maintenance MMF and corticosteroids. The trial was converted into an exploratory study without sufficient power to address its primary and secondary objectives because of four cases of BK virus nephropathy (BKVN) occurring in CP30 group. The CP15 group had a similar rejection rate when compared to the Tac group. The CP30 group had a higher rejection rate than the Tac group and the combination of JAK3 inhibition with MMF therapy yielded excessive viral opportunistic infections such as BKVN and CMV disease.

Voclosporin (ISA247)

Voclosporin is a modified CNi with an additional carbon molecule attached to the amino acid 1 residue of CsA resulting in more potency and less toxicities. A phase IIB multicenter study, conducted on de novo kidney transplant recipients, showed a lower incidence of new-onset diabetes after transplantation in patients on voclosporin and the incidence of biopsy-proven acute rejection was not inferior in voclosporin arms compared to Tac arm (Busque et al. 2011).

TOL101

TOL101, an orphan drug, is a murine mAb directed against the $\alpha\beta$ TCR. Unlike anti-CD3 antibodies, which may activate intracellular im-

munoreceptor tyrosine-based activation motifs, anti- $\alpha\beta$ TCR antibodies do not, thus potentially limiting drug toxicity (Getts et al. 2010). Selectively targeting $\alpha\beta$ T cells using TOL101 may also preserve the role of $\gamma\delta$ T cells.

CONCLUSIONS

Sixty years after the pioneering tolerance studies of Medawar and his colleagues (Billingham et al. 1953), immune rejection continues to be a dreaded complication. The emergence of novel agents with efficacy not only against the cellular, but also the humoral arms of the immune response may improve the management of transplant recipients. A one-size-fits-all approach remains the clinical norm despite serious toxicities. A personalized immunosuppressive drug therapy based on mechanistic biomarkers remains an unmet objective and worthy of pursuit.

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Immunosuppressive Drug Therapy

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