Tolerance—Is It Worth It?

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We are entering an exciting time in the study of immunologic tolerance. Several cellular and molecular strategies have been developed that show promise in nonhuman transplant models and these approaches are just now appearing in clinical trials. Tolerance strategies that prevent immune rejection and obviate the need for immunosuppressive medications (with inherent risk of cancer, infection, and organ toxicity) would improve both graft and patient survival. Each tolerance protocol brings its own set of associated risks. As the results of these trials become available, we must continue to evaluate their successes and failures. The balance of these outcomes will help us answer the question: “Tolerance—Is it worth it?”

WHAT IS TOLERANCE?

In the words of Nobel Prize winner Peter Medawar, “‘Immunological tolerance’ may be described as a state of indifference or non-reactivity towards a substance that would normally be expected to excite an immunological response” (Medawar 1964). The concept of tolerance is further clarified by the editors of the *American Journal of Transplantation* as “a durable state of antigen-specific unresponsiveness, induced by exposure to antigen, in a patient who is otherwise fully immunologically competent” (Halloran et al. 2008).

The experimental evidence for tolerance began with the 1945 studies of Owen (1945) who showed persistence of postnatal red blood cell chimerism in freemartin cattle that share a common placenta and develop cross-placental common circulation. The significance of these

observations to transplantation was made apparent by Billingham, Medawar, and colleagues, who showed tolerance to skin grafts from non-identical dizygotic cattle twins (Anderson et al. 1951; Billingham et al. 1952). Interestingly, these observations occurred in a failed attempt to develop a technique to distinguish monozygotic versus dizygotic twins. The original hypothesis was that skin grafts between dizygotic twins would reject, whereas grafts between single-cell twins would not. To the contrary, skin grafts from both identical and dizygotic twins were accepted without rejection. Similar findings were shown by parabiosis in birds, in which embryonic cross circulation made siblings incapable of making antibodies against each other, or of rejecting skin grafts (Hasek and Hrabá 1955).

Billingham et al. (1953) reported the first formal documentation of transplant tolerance

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by demonstrating that inoculation of fetal mice or chickens with donor cells of a different strain of that species made these animals tolerant to skin grafts of the donor strain. This study is the first report of tolerance induction by mixed chimerism. However, there has been some debate as to whether these experiments show true tolerance (Halloran et al. 2008). Subsequent studies suggest that mixed chimerism may, in itself, be a form of immunosuppression, as this antigen's nonresponsiveness was not donor-specific (Hofmann et al. 2004). There is disagreement over the interpretation of these findings (Sachs et al. 2008). Additional evidence shows persistence of intact immune function remains with mixed chimerism (Ildstad et al. 1985; Rüedi et al. 1989), and that although nonresponsiveness in these studies was not strain specific, they were at least organ specific (Hofmann et al. 2004).

Despite conflicts in the formal definition of tolerance, a more practical definition may be that of operational tolerance. This may be defined as "a well-functioning graft lacking histological signs of rejection, in the absence of any immunosuppressive drugs (for at least 1 year), in an immunocompetent host" (Ashton-Chess et al. 2007; Orlando et al. 2010). Operational tolerance may further be clarified to include the absence of antibody-mediated rejection.

WHY IS TOLERANCE IMPORTANT?

Transplantation is the best, and in some cases only, treatment for many types of end-stage organ disease (Grinyó 2013). It has been shown to increase both quality and length of life for those affected by organ failure. Unfortunately, for the vast majority of transplant recipients there is an obligate requirement for lifelong immunosuppression. These immunosuppressive medications increase the risk of cancer, infection, and direct drug-associated organ toxicity, all of which contribute to reduced patient survival.

The most common cause of death in patients with functioning renal transplants is cardiovascular disease (24%), followed by infection (16%), and malignancy (12%) (Kahwaji et al. 2011). Each organ has a different distribution of causes of death after transplant, but in-

fection and cancer are frequent etiologies in all (Chapman et al. 2013; Fishman 2013a). There is an ~50% overall risk of developing some form of cancer (including nonmelanoma skin cancer) attributed to immunosuppressive medications (Scherer et al. 2007). The association of individual cancers with immunosuppression varies, but relative risks of two- to 20-fold have been reported (Kasiske et al. 2004) with the greatest risk being non-Hodgkin's lymphomas and nonmelanoma skin cancers. The risks of Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) vary based on organ type, age at transplant, and EBV serostatus. A recent report from a large kidney transplant series measured annual incidence of 2.6 cases of PTLD per 1000 patient years and an increase in Hodgkins and non-Hodgkins lymphoma of 5.9 and 7.6 times, respectively, the rate in the general population (Morton et al. 2013).

Transplant patients are also at increased risk for both routine and opportunistic infections (Fishman 2007, 2013a). Of particular concern is the increased susceptibility to viral and fungal infections (Fishman 2002, 2013b; Winston et al. 1995; Winston and Busuttill 2002). Opportunistic infections are even more prevalent in patients undergoing antilymphocyte induction therapy (Issa and Fishman 2009). Infection is second only to cardiovascular disease, which is one of the most common causes of death in kidney transplant recipients (Djamali et al. 2006).

Immunosuppressive agents have a range of associated direct toxicities. Of particular concern is chronic nephropathy of the transplanted allograft in renal transplant recipients or the native kidneys in extrarenal transplant recipients (Ojo et al. 2003). New onset diabetes associated with calcineurin inhibitors is a well-described complication (Kasiske et al. 2003). With strict definition for new onset diabetes, the tacrolimus-associated incidence is in excess of 30% in kidney recipients and even higher in liver recipients (First et al. 2013). The degree of posttransplant hyperglycemia is predictive of cardiovascular and all-cause mortality (Valderhaug et al. 2011).

In addition to the adverse health effects of these medications, there is a continued problem of graft loss due to noncompliance. A review of multiple reports of compliance in kidney transplantation reported that approximately 22% of patients are noncompliant with their medications (Butler et al. 2004). Furthermore, 36% of graft losses are associated with prior noncompliance, and the odds ratio of graft failure is sevenfold higher for nonadherent patients (Butler et al. 2004). A report from Sweden documented that 16% of renal transplant recipients report having missed doses of immunosuppression in the preceding month (Lennertling and Forsberg 2012). The problem is even more pronounced in pediatric recipients, in which there is as much as a 75% nonadherence rate (Fredericks and Dore-Stites 2010). In kidney transplantation, long-term mortality rate was 48%–82% lower among transplant recipients than patients on the waiting list (Wolfe et al. 1999). Graft losses due to noncompliance or direct toxicity of these medications likely contribute to overall shortened patient lifespan.

Successful induction of transplant tolerance would eliminate immune rejection. Even with continued use of therapeutic immunosuppression, graft loss due to immune-mediated acute and chronic rejection shortens both graft and patient survival. Since the first successful kidney transplant in 1954 (Harrison et al. 1956), there has been a continuous improvement in transplant outcomes for all organ types (Organ Procurement 2012). Despite improvements in predominantly short-term outcomes over the last 50 years, improvements in chronic graft loss have not kept pace (Meier-Kriesche et al. 2004; Sykes 2007). Even in the absence of organ dysfunction, there can be subclinical rejection present in nearly half of biopsy specimens. Over time, severe chronic allograft nephropathy develops in 58.4% of patients (Nankivell et al. 2003). This damage, once established, is irreversible, resulting in declining renal function and graft failure. This resulting decline in glomerular filtration rate (GFR) is predictive of mortality following renal transplant (Moranne et al. 2013). In liver, lung, and heart transplants,

immune-mediated graft loss leads to patient death unless retransplant is possible.

Tolerogenic strategies that lessen infectious complications, reduce cancer risk, avoid direct pharmacologic toxicity, and prevent graft loss due to acute and chronic immune destruction would improve graft and subsequent patient survival.

HOW CAN TOLERANCE BE ACHIEVED?

Many approaches have been pursued with the objective of tolerance in solid organ transplantation. These approaches can be divided into three main groups: (1) study of noncompliant patients that show spontaneous tolerance following discontinuation of immunosuppression, (2) patients with stable long-term graft function that undergo planned immunosuppression minimization and cessation, and (3) pretransplant-planned protocols for tolerance induction via pharmacologic or cellular therapy (Orlando et al. 2010). There are examples of successful withdrawal of immunosuppression with each of these approaches. Although they may not meet the strict definition of “tolerance,” they fit within a more clinically relevant description of “clinical operational tolerance.” Some of the most promising approaches are summarized below.

Study of Spontaneous Tolerance

There have been many reports of sporadic tolerance identified in noncompliant patients (summarized by Orlando et al. 2010). It is clear that organs differ in their response to cessation of immunosuppression out of noncompliance. There have been many reports of tolerance in kidney transplantation (Orlando et al. 2010), a single report in lung transplant (Di Cocco et al. 2009), and no reports in pancreas transplant. Liver transplant recipients are most likely to have continued graft function after cessation of immunosuppression. Multiple reports document durable liver allograft function after discontinuation by nonadherence (Orlando et al. 2009). There are other case reports or small series of tolerance following discontinuation un-

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der circumstances other than noncompliance. One report documented operational tolerance in heart transplant after total lymphoid irradiation for resistant rejection and subsequent withdrawal of all medications, including immunosuppression, because of drug intolerance (Comerci et al. 2009). Similar findings have been found in renal transplants with operational tolerance after total lymphoid irradiation (Strober et al. 1989, 2000). A number of case reports have documented spontaneous tolerance in a subset of patients undergoing immunosuppression withdrawal for treatment of PTLD (Orlando et al. 2010).

Planned Immunosuppression Weaning

Based on the findings of spontaneous tolerance in nonadherent patients, several studies have been performed that sought to wean patients off of immunosuppression as a planned treatment protocol. Orlando reviewed reports of operational tolerance in kidney (Orlando et al. 2010) and liver transplantation (Orlando et al. 2009) following planned immunosuppression withdrawal. In liver transplant, there is an approximate 22% success rate for protocols of withdrawal. In a particularly informative study in liver transplantation, it was found that pediatric patients may be uniquely amenable to immunosuppression weaning (Feng et al. 2012). In this study of planned immunosuppression weaning in patients with stable graft function on single agents after liver transplant for non-viral and nonautoimmune etiologies of ESLD, there was a 60% success rate in immunosuppression withdrawal.

The prevalence of potentially tolerant renal transplant recipients is clearly less than in liver transplant recipients, but the actual percentage of patients is harder to define. Most studies rely on a series of case reports (Roussey-Kesler et al. 2006) or assembled groups of patients already with stable graft function after nonadherence. Approximately 100 cases of operational tolerance in renal transplant have been reported (Orlando et al. 2010) with a denominator of many tens of thousands of total kidney transplants. There are a steadily increasing number of re-

ports of different immune monitoring phenotypes or “signatures” that characterize tolerant recipients (Brouard et al. 2005, 2007; Newell and Larsen 2006; Einecke et al. 2010; Newell et al. 2010; Sagoo et al. 2010). It may be possible to use these combined signatures to identify candidates for immunosuppression weaning.

Pretransplant Tolerance Protocols

Mixed Chimerism

A promising approach developed by several groups makes use of strategies to induce mixed chimerism (Sachs et al. 2013). This is accomplished by a stem-cell infusion in combination with a transplant from the same donor. There are several variations to this approach. The earliest reports were in patients that first had bone marrow/stem cell transplantation and then later had a kidney transplant from the same donor (Sayegh et al. 1991; Helg et al. 1994; Jacobsen et al. 1994; Sorof et al. 1995; Butcher et al. 1999; Sellers et al. 2001; Light et al. 2002). Operational tolerance was reported in these recipients. Subsequent studies have documented some degree of success with a combined or simultaneous stem cell and kidney transplant from the same donor rather than the earlier sequential approach.

The group from Massachusetts General Hospital (MGH) showed tolerance in six patients undergoing simultaneous bone marrow and kidney transplants from human leukocyte antigen (HLA)-identical donors for the treatment of multiple myeloma in patients with renal failure (Fudaba et al. 2006). Three patients showed transient donor chimerism, but were successfully weaned off immunosuppression. Two patients had full chimerism, but immunosuppression was resumed for treatment of graft-versus-host disease (GVHD). One patient developed acute rejection and was treated with resumption of cyclosporine, but was later able to be weaned off immunosuppression once again.

This report was followed by a more rigorous non-HLA-identical series from the same group wherein they transplanted five patients with single haplotype mismatched sibling donors (Kawai et al. 2008). They performed a pretransplant

nonmyeloablative conditioning protocol with cyclophosphamide, thymic irradiation, and humanized anti-CD2 monoclonal antibody. Induction therapy also consisted of short-course cyclosporine or tacrolimus (plus rituximab and steroids in a subset of patients). Four out of five patients showed operational tolerance and were weaned off of immunosuppression. The fifth developed early refractory antibody-mediated rejection and had graft failure. The tolerant patients showed transient microchimerism and persisting donor-specific hyporesponsiveness in *in vitro* assays (Kawai et al. 2008; LoCascio et al. 2010). In subsequent follow up, there were three cases of acute or chronic GVHD, for which two recipients were returned to immunosuppression (Spitzer et al. 2011). Three patients had recurrence of multiple myeloma. This trial has since been extended to 10 patients; seven have shown long-term graft survival without maintenance immunosuppression (Markmann and Kawai 2012). Three failures occurred after withdrawal of immunosuppression from early humoral rejection, thrombotic microangiopathy, and acute rejection.

A group from Stanford reported on their series of 16 patients undergoing simultaneous HLA-identical kidney transplant and same-donor granulocyte-colony stimulating factor (G-CSF)-mobilized CD34⁺ stem-cell infusion (Scandling et al. 2012). They used a nonmyeloablative conditioning regimen of total lymphoid irradiation and antithymocyte globulin. Fifteen out of 16 developed mixed donor/recipient chimerism. Eight of these showed chimerism for more than 6 months and were weaned off immunosuppression. Four patients were undergoing immunosuppression wean. Four patients developed recurrent disease or rejection and were not weaned. All patients had functioning grafts at the end of the reporting period and there was no GVHD.

The groups from Northwestern and University of Louisville reported a novel approach to augment donor stem cell engraftment in conjunction with renal transplant (Leventhal et al. 2012). They make use of a bone marrow-derived CD8⁺, TCR[−] “facilitator cell” population that promotes the engraftment of allogeneic hema-

topoietic stem cells in animal models without observed GVHD (Kaufman et al. 1994). To date, data on 15 patients undergoing their protocol have been reported (Leventhal et al. 2012, 2013). Recipients undergo a “low-intensity” conditioning regimen consisting of fludarabine, cyclophosphamide, and 200 cGy total-body irradiation. Subsequently, a combined HLA-mismatched renal transplant with same-donor hematopoietic stem-cell and facilitator-cell infusion is performed. Fourteen out of 15 showed initial macrochimerism that was eventually lost in three patients. There was one engraftment failure in a patient with panel reactive antibody (PRA) of 52%. Initial immunosuppression was tacrolimus and mycophenolate mofetil, which was successfully weaned off at 1 year in all patients with durable macrochimerism (12 patients). One patient lost their graft because of renal artery thrombosis after viral infection and sepsis. There were no cases of GVHD or engraftment syndrome. Of note, all patients that were successfully weaned off all immunosuppression had extremely high levels of donor-cell engraftment (94%–100% donor type), suggesting donor-type replacement rather than mixed chimerism. Two concerns with these reports were the lack of details on the engineered facilitator population and a lack of a control group without facilitator cells (Markmann and Kawai 2012). However, in a similar induction therapy for stem cell transplant, there was an engraftment failure rate of 13% and severe GVHD in 6% (Luznik et al. 2008; Markmann and Kawai 2012).

Regulatory T Cells

A different cellular therapy approach uses regulatory T cells (Treg) (Cobbold and Waldmann 2013). These lymphocytes develop in the thymus (natural Treg) or are induced in the periphery in response to antigen exposure in the presence of TGF- β (adaptive Treg) (reviewed in Josefowicz et al. 2012). Treg are phenotypically described by their expression of the transcription factor FoxP3. When these cells encounter their cognate antigens properly presented by antigen-presenting cells, they suppress the activation and function of nearby effector T cells

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(Schmidt et al. 2012). Treg are important in a wide variety of counterinflammatory conditions and are a promising target for tolerance-induction strategies.

The first clinical use of Tregs has been in the prevention or treatment of GVHD. Trzonkowski et al. (2009) performed a trial in two patients who were failing standard treatment of GVHD following allogeneic stem cell transplant. Treg were isolated from the stem cell donor and infused back into the recipient. In one patient with chronic GVHD, they were able to stabilize symptoms and wean immunosuppression medications. The long-term durability was not reported. In a second patient with acute GVHD, they were able to temporize symptoms with repeated dosing of Treg. However, they ran out of donor material, were unable to infuse further cells, and the patient relapsed and succumbed to the disease.

A second trial reported by Di Ianni et al. (2011), attempted to use Treg paired with conventional T-cell infusion to prevent GVHD in patients at high risk for GVHD, and to more quickly restore cellular immunity following stem cell transplant. This study consisted of 28 patients with high-risk hematologic malignancies that underwent haploidentical hematopoietic stem cell transplant. The recipients underwent induction conditioning with total-body irradiation and treatment with thiopeta, fludarabine, and cyclophosphamide. G-CSF-mobilized, CD34-enriched hematopoietic stem cells from the donors were infused along with ex vivo expanded Treg and conventional T cells. Twenty-six out of 28 had sustained full donor-type engraftment. Two out of 26 developed grade 2, or better, acute GVHD. No chronic GVHD was observed and there was improved T-cell reconstitution and decreased cytomegalovirus compared with standard hematopoietic stem cell transplantation (HST).

A third trial was performed at the University of Minnesota (Brunstein et al. 2011). This study was a phase I dose escalation trial of ex vivo expanded umbilical cord Treg. Patients underwent a conditioning regimen consisting of total-body irradiation and treatment with fludarabine and cyclophosphamide. Patients received

two partially HLA-matched umbilical cord blood infusions followed by infusion of Treg isolated and expanded from a third umbilical cord blood sample that was 4-6/6 HLA matched to the patient. Induction immunosuppression was cyclosporine and mycophenolate mofetil (MMF) in the first group of patients and then sirolimus and MMF in the second group. There was no increased risk of infection, relapse of disease, or mortality. There was a decrease in severe GVHD from 61% to 43%.

In an interesting but theoretically contradictory approach, trials are beginning that combine rapamycin and IL-2 administration in diabetes, GVHD, and composite tissue transplants. This combination has a number of Treg-friendly benefits. Treg are IL-2 dependent and IL-2 rather surprisingly serves as a death factor for CD8⁺ memory T cells (Ku et al. 2000) or newly activated effector T cells (Li et al. 2001) by opposing cytokines. Low-dose IL-2 administration in diabetes-prone nonobese diabetic (NOD) mice promotes Treg survival and protects against the development of diabetes (Tang et al. 2008). The combination of IL-2 and rapamycin favor Treg survival and T effector apoptosis by activating T cells toward an apoptotic pathway rather than clonal expansion (Rabinovitch et al. 2002).

Triple therapy with doses of IL-2Ig that saturate the high affinity, but not intermediate affinity IL-2 receptor, mutant antagonist type IL-15Ig, and rapamycin tilts the balance of immunity from rejection to or toward tolerance in a variety of daunting murine transplant (Zheng et al. 2003) and new onset type 1 diabetes models (Koulmanda et al. 2007). Triple therapy also results in long-term islet allograft survival in nonhuman primates (Koulmanda et al. 2012). The mechanism is hypothesized to be selective depletion of alloreactive T cells and stabilization/expansion of Tregs. Recent clinical trials of low-dose IL-2 therapy in corticosteroid-resistant GVHD (Koreth et al. 2011) and virus-induced vasculitis (Saadoun et al. 2011) have produced exciting results, whereas higher doses produce toxicity by activating innate immune cells bearing the intermediate affinity IL-2 receptor despite expansion of Tregs.

Currently, there are several trials of Treg in solid organ transplantation under way, including a large multinational trial (www.onestudy.org) and other center-based trials. Although there is considerable hope that Treg may be important in the induction of tolerance, many questions persist. How many Treg are needed to prevent rejection? What is the source of Treg? Donor cells? Recipient cells? Do Treg need to be antigen-specific, or would polyclonal expanded cells be sufficient? How long do these cells last once infused? Based on experimental studies in animal models, there may be some benefit to combining Treg with stem-cell infusion. As has been seen with facilitator cells and hematopoietic stem cells, Treg may help with establishment of mixed chimerism and prevent induction of GVHD. Thus, the Treg themselves may not be tolerogenic, but they may support persistence of mixed chimerism that induces tolerant phenotype.

Costimulatory Blockade

Costimulatory molecules provide another attractive target for tolerance-induction protocols (Maltzman and Turka 2013). There are abundant examples in mice in which a blockade of costimulatory molecules prolongs graft survival (reviewed in Kinnear et al. 2013). The three main pathways that have approached as clinical targets have been the CD28:CD80/CD86, CD40:CD154, and LFA-1:ICAM receptor:ligand pairings.

The CD28 costimulatory pathway was the first of these pathways to have a biologic agent approved for clinical use in transplant. In murine experimental models, CTLA-4Ig administration leads to prolonged or indefinite graft survival (Lenschow et al. 1992; Baliga et al. 1994; Pearson et al. 1994, 1997; Judge et al. 1996). CTLA-4Ig works via two pathways to inhibit alloreactive T-cell function. First, CTLA-4Ig binds to CD80 and CD86 on antigen-presenting cells and inhibits binding of these ligands to CD28 molecules on T cells. This inhibition reduces CD28-mediated costimulation during antigen recognition. Second, CTLA-4Ig binding to CD80/86 molecules also induces

indoleamine 2,3-dioxygenase (IDO) expression in dendritic cells via an IFN- γ -dependent pathway. Up-regulation of IDO leads to changes in tryptophan metabolism that suppresses T-cell function (Grohmann et al. 2002).

In 2011, belatacept (human CTLA-4Ig) was approved by the Food and Drug Administration for use in kidney transplantation for the prevention of acute rejection in combination with basiliximab, mycophenolate mofetil, and corticosteroids. A number of trials have reported the outcomes of belatacept-based immunosuppression protocols in comparison to cyclosporine-based regimens. One- and 3-year outcomes have shown similar graft and patient survival between cyclosporine and belatacept protocols, but slightly more rejection with belatacept (Durrbach et al. 2010; Vincenti et al. 2010; Rostaing et al. 2011; Pestana et al. 2012). However, belatacept-treated patients had better preservation of kidney function with a higher GFR than the cyclosporine-treated patients. In diabetic patients, there was an increase in patient and graft survival with belatacept, but this did not achieve statistical significance (Rostaing et al. 2011). In each of the studies, there was an increased risk of PTLD with belatacept. However, this was seen predominantly in EBV-naïve patients. Currently, belatacept is not recommended for use in transplant recipients who are EBV negative. In the 3-year follow up, there was an increase in the incidence of tuberculosis infection (Pestana et al. 2012).

A second potential target for costimulatory blockade is the CD40:CD154 pathway. Blockade of this pathway results in prolongation of transplant survival in mouse (Parker et al. 1995; Hancock et al. 1996; Larsen et al. 1996; Larsen and Pearson 1997; Shimizu et al. 2000) and nonhuman primate models (Kenyon et al. 1999; Kirk et al. 1999; Koulmanda et al. 2006). However, clinical trials in humans were halted because of safety concerns over thromboembolic events (Sidiropoulos and Boumpas 2004; Couzin 2005). These thromboembolic events were validated in nonhuman primate models and the mechanism was postulated to be caused by unanticipated binding to CD154 on vascular endothelium and platelets (Kawai et al. 2000). Although

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most preclinical studies reveal anti-CD154 to be more potent than most anti-CD40 mAbs as immune therapy, Reimann has developed an anti-CD40 mAb with immune-inhibitory functions as evidenced by the ability to block IgG production by lipopolysaccharide-activated B cells. This unique antibody has enabled drug-free nonhuman primate islet allograft survival, suggesting that optimization of this approach may have great promise (Lowe et al. 2012).

The third target, efalizumab, is a humanized anti-LFA-1 (CD11a/CD18) monoclonal antibody that was approved for the treatment of psoriasis (Dedrick et al. 2002; Stern 2003), and has been effective at reducing the severity of the disease in moderate to severe psoriasis (Gordon et al. 2003; Lebwohl et al. 2003). The antibody blocks the cell-adhesion molecule LFA-1 from binding to its ligand ICAM-1. LFA-1-mediated cell adhesion is important in a number of steps in the immune process including leukocyte adhesion to endothelium and the strengthening of T-cell/antigen-presenting cell interactions (Dedrick et al. 2002). Anti-LFA-1 was tested in a clinical trial in kidney transplant recipients (Vincenti et al. 2007). It was used in combination with cyclosporine, MMF, and prednisone. Although graft and patient survival was excellent (95% and 97% at 6 months), there was an 11% rejection risk and an 8% PTLD incidence. Other groups have used efalizumab as adjunct therapy in islet transplant trials (Posselt et al. 2010; Turgeon et al. 2010) as part of a maintenance immunosuppression regimen. The use of this agent was halted because of an association with progressive multifocal leukoencephalopathy (PML), a serious and usually fatal central nervous system infection caused by JC polyoma virus (Carson et al. 2009). However, PML has also been associated with other immunosuppressive mediations that remain in common use (Neff et al. 2008; Carson et al. 2009; Weber et al. 2011).

Other Possibilities

A number of additional cellular therapy approaches with promising results in preclinical studies are entering into clinical trial (reviewed

in Page et al. 2012; Tang and Bluestone 2013). Tolerogenic macrophage, dendritic cells, and mesenchymal stromal cells have all shown promise in transplant models. Transplant acceptance-inducing cells are a form of regulatory macrophage. Two trials using infusion of donor-derived splenic monocytes in kidney transplant were performed (Hutchinson et al. 2008a,b). Standard immunosuppression induction was initiated and patients were weaned to low-dose monotherapy (or completely off of immunosuppression in one patient). There was an elevated incidence of early acute rejection, but several patients were able to wean down to low-dose single agent. Additional cell types with tolerogenic properties include mesenchymal stromal cells (English and Wood 2013) and tolerogenic dendritic cells (Page et al. 2012). These cell types have been shown effective in animal models and are also entering into clinical testing.

AT WHAT COST?

Highlighted above are a number of approaches that have been, or are soon to be, put into clinical trials as potentially tolerogenic therapies in transplantation. Despite some promising outcomes with several of these approaches, they each pose risk of adverse events and outcomes. For each approach there are specific complications, but these may represent more generalized concerns.

Risks of Stem Cell Infusion

A particular concern of the mixed chimerism approaches is the toxicity of the induction protocols. These protocols make use of chemotherapeutic medications and systemic irradiation as part as their engraftment protocol. These treatments come with both short- and long-term sequella. Although the immediate health outcomes reported seem to decrease with expected outcomes after transplant, we do not know the long-term health impact. An additional risk is the development of GVHD, a potentially life-threatening outcome, which was seen in several of the combined hematopoietic stem cell transplantation (HSCT)/kidney recipients.

Although we do not have long-term outcomes on all patients in the mixed chimerism trials, we can look at similar protocols for HSCT treatment of hematologic malignancies. Apart from recurrent disease, toxicity of the induction protocols and GVHD are the biggest long-term risk factors for these patients (Tichelli et al. 2009, 2012). HSCT recipients have increased risk of endocrine, musculoskeletal, and cardiovascular disorders in addition to increased risk of malignancy. These factors have an impact on morbidity and mortality following treatment. Patients alive at 5 years posttransplant still have a 30% reduction in life expectancy compared with the general population (Bhatia 2011).

Risks of PTLD

The risk of PTLD is increased in several of the trials described, particularly with the use of belatacept and efalizumab. This risk may be inherent to the tolerance approach, as the recipient's immune system is less able to fight off the subset of PTLDs that are donor cell in origin (Olagne et al. 2011). The concern of PTLD is greatest in patients who are EBV-naïve. Avoiding this population of patients may mitigate some of these concerns.

Off-Target Effects

A particular concern in trials of Treg is whether the suppression of allogeneic immune responses will lead to suppression of other off-target immune functions. That is, will Tregs that suppress rejection of liver transplant also suppress antiviral responses to the hepatitis virus that caused the disease (Dolganiuc and Szabo 2008)? Or will they suppress antitumor responses to hepatocellular carcinoma present in the liver explant (Lin et al. 2013)? We know that Treg are capable of bystander suppression (Tang and Bluestone 2008). We do not know if this suppression would be of harm to the transplant recipient (or maybe of benefit if the disease was an autoimmune process).

Other unanticipated off-target effects were seen with costimulation blockade trials. Anti-

bodies against CD154 resulted in increased thromboembolic events. The unexpected complication was likely due to expression of this molecule on unanticipated cell types (platelets and endothelial cells). Other agents that block CD40 may have similar efficacy, but avoid these complications. Use of efalizumab was halted when there was a concern for increased risk of PML. This risk may be common to many immunosuppressant protocols, but serves as an example of another unexpected off-target effect.

Economic Considerations

A final consideration applicable to all of these approaches is an economic one. These protocols are expensive. Each protocol costs several million dollars to enroll a limited number of patients. In our own center, the cost of Treg isolation and expansion alone is ~\$45 K per transplant. Counterbalancing this cost are the expenses of traditional transplants. Financial analysis is most complete for end-stage renal disease (ESRD) and renal transplantation. Data from the USRDS (2012) report shows that the average Medicare cost of ESRD in 2010 was \$87,561 per person per year. Despite the cost of the transplant procedure, it is estimated that the transplant-associated savings are approximately \$200,000 over 5 years (reviewed in Axelrod 2013). The cost of the transplant procedures themselves would likely be similar for standard therapy and tolerance protocols. The economic benefits of tolerance protocols would be generated by reduction in immunosuppression costs. Annual Medicare costs for injectable medications and immunosuppression following transplant was \$10,000 in 2010 (USRDS 2012). Additionally, there would be significant cost savings by potentially avoiding costs associated with long-term viral and bacterial prophylaxis and treatment of opportunistic infections. Tolerance approaches could be financially advantageous if the cost savings associated with avoiding immunosuppression and chemoprophylaxis increased graft survival, and reduced need for retransplants or dialysis exceeds the additional costs of these procedures.

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CONCLUSIONS

It is clear that transplantation improves quality and length of life for patients with end-stage organ diseases. Unfortunately, the vast majority of transplant recipients require life-long immunosuppression. This immunosuppression increases risk for cancer, infection, and other direct organ toxicity. Despite continued use of these medications, functional organ lifespans are finite. There is a persisting risk of immune and nonimmune-mediated graft loss that results in decreased patient survival. Strategies that enable long-lasting graft function obviate need for immunosuppression medication, and maintained immune function could increase patient longevity. The question remains, are the costs of these tolerance-induction protocols worth the benefits of immunosuppression avoidance?

There are many considerations when attempting to answer “Is it worth it?” These considerations may not be the same for all organ systems or even for different recipients. For example, it may be worth the risks of total body irradiation and GVHD present in mixed chimerism protocols for a 50-year-old who gets a heart transplant and may not be a candidate for retransplant should their heart fail at the age of 60 from chronic rejection. On the other hand, a 25-year-old living donor kidney recipient might not want to take those risks if they know that if their graft fails they have the option of dialysis, and perhaps have another potential live donor for that eventuality. The consequences of transplant failure for life-saving organs (heart, lung, and liver) may make these recipients more suitable for higher-risk tolerance protocols than for organs with alternative therapies (insulin for pancreas transplants and dialysis for kidney transplants). Additional factors may influence the risk/benefit balance. Highly sensitized patients with donor-specific anti-HLA antibodies may take the risk of a tolerance protocol given their likely reduced graft survival compared with an unsensitized recipient receiving a 0-HLA mismatch graft. It may be one thing to ask a patient with hematologic malignancy to undergo a risky combined stem cell and kidney

transplant with irradiation and chemotherapy, but a completely different consideration for a relatively healthy person needing a preemptive kidney transplant. Clearly, each tolerance protocol has a set of risks that must be balanced with the long-term benefits. This risk/benefit analysis would need to be individualized to the organ type, disease process, and even individual patient circumstances, and would also depend on the efficacy and costs of the protocol developed.

The last consideration is: What are the consequences of failure? Under current practice, transplant outcomes are pretty good (at least for short- and medium-term outcomes). Each of the tolerance protocols will have some failure rate. Is the long-term benefit in the successes worth the risks of graft loss, patient death, or other complications in the failures?

The evaluation of each tolerance protocol will be influenced by the success rates and the implications of failure. If protocol failure means loss of graft and need for retransplant in a kidney recipient, the risk may be warranted. If failure means loss of graft and death in a heart transplant recipient, the considerations will be different. If failure means resumption of immunosuppression, the down side is low. If protocol failure means graft loss or death, the implications are more considerable. For example, in the MGH series of patients undergoing combined bone marrow and kidney transplant for multiple myeloma, seven out of 10 patients were successfully weaned off of immunosuppression with stable long-term graft function (Markmann and Kawai 2012). However, three out of 10 failed their protocol and lost their grafts. Is a 70% long-term survival rate free of immunosuppression worth the 30% risk of graft loss? Also, what do we mean by “long-term graft survival?” The MGH experience has shown us that success measured at 1 year may lead to failures after five. Each organ, disease, and patient will have differing answers to these questions.

The answer to “Tolerance—Is it worth it?” is clear—“It depends.” The up side of a nontoxic protocol that enables long-term graft and patient survival without immunosuppression would be tremendous. But currently the risks

are significant. As more results of ongoing and future trials become available, we must continually evaluate what are our successes and what are our failures? The decision of “Is it worth it” depends on the balance of the two.

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