Past, Present, and Future Prospects for Inducing Donor-Specific Transplantation Tolerance for Composite Tissue Allotransplantation

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ABSTRACT

Composite tissue allotransplantation (CTA) is among the most immunologically complex and newest transplant fields. Although the field has made considerable advances, there are still concerns that these procedures are performed to enhance quality-of-life issues and are not lifesaving procedures that restore physiologic function. Two challenges limit the widespread application of CTA; the first is chronic rejection, the most prevailing cause of organ allograft failure after transplantation; the second barrier is the numerous health complications associated with lifelong immunosuppressive therapy. Several tolerance-inducing strategies, including costimulatory blockade, T-cell depletion, mixed chimerism, and gene targeting of transplanted organs, have the potential to induce lifelong tolerance to organ allografts without chronic immunosuppression. Effective clinical tolerance protocols that improve CTA acceptance and offer an alternative to the requirement for chronic immunosuppressive therapy could be a major advance in the field. Tolerance would allow allotransplantation to provide a currently unmet need for reconstruction of large tissue defects. This article reviews the history of CTA, current challenges and complications, and offers future directions for CTA research in strategies to induce tolerance.

KEYWORDS: Composite tissue allotransplantation (CTA), immunosuppression, tolerance, induction immunosuppression, maintenance immunosuppression

Although significant improvements have been made in composite tissue allotransplantation (CTA) in the past 20 years, the full realization of the potential has in some degree been limited. Whereas solid organ transplantations restore physiologic function and are life-saving, CTA facilitates the restoration of function and structural reliability and is considered a quality-of-life intervention. Because CTA recipients for the most part have non-life-threatening illnesses, the major ethical

concern and an obstacle is the exposure of CTA recipients to lifelong immunosuppressive therapies and their associated and significant side effects.¹ To completely comprehend the existing status of CTA, it is essential to understand the historical events in the advancement of the field. This article reviews the historical facets of CTA, discusses the current immunosuppressive protocols, complications associated with CTA, and speculates on future directions for CTA research. The elusive goal

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Composite Tissue Allograft Transplantation; Guest Editor, Maria Siemionow, M.D., Ph.D., D.Sc.

Semin Plast Surg 2007;21:213–225. Copyright © 2007 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.

DOI 10.1055/s-2007-991191. ISSN 1535-2188.

Figure 1 Master of Los Balbases (ca. 1495): The Miracle of Cosmas and Damian.

of establishing donor-specific tolerance would significantly impact the widespread use of CTA.

The use of CTA for the restoration of inherited or acquired deformities is not a novel procedure. The earliest known account of allograft transplantation dates back to AD 348 in which two sainted twin brothers, Cosmas and Damian, were charged with replacing the gangrenous, cancerous leg of an elder church sacristan.² As the sacristan slept, the two doctors replaced the diseased leg with that of a recently deceased Ethiopian Moor (Fig. 1). The sacristan awoke to discover his tumorous leg amputated and replaced with a healthy one. Then, during the 16th century, Gaspare Tagliacozzi, the man whom many consider the "Father of Plastic Surgery," used an allogeneic flap of tissue to reconstruct a man's nose sheared off during swordplay. The reconstructed allogeneic nose survived for 3 years until it rejected suddenly. According to the story, the slave who donated the tissue from his arm for the surgery died at the same time as the rejected graft.¹ Tagliacozzi concluded that the allograft was rejected as a result of difficulties in "binding" tissues together from two disparate individuals for an adequate amount of time.³

HISTORY OF HUMAN COMPOSITE TISSUE ALLOTRANSPLANTATION

For the past 30 years, many forms of CTA have been performed. In addition to enhanced microvascular surgical techniques and the transplantation of complex tissue combinations, our understanding of immune responses to allografts has advanced substantially. As a result, there has been an evolution in the immunosuppressive treatment protocols for composite tissue allografts. Strategies to one day achieve lifelong tolerance to grafts without the need for nonspecific or prolonged

immunosuppression are being intensively pursued. CTA has benefited significantly throughout the years by data acquired from these initial studies that ultimately gave rise to the recent hand transplants.

Hand Allotransplantation

Hand transplantation has been an amazing success story. The first hand transplant was performed in 1964 in Ecuador.⁴ The patient was given first-generation immunosuppressive drugs, which included steroids and azathioprine to avoid rejection. The hand was rejected after 2 weeks. Little was ascertained from that operation because of limited immunologic testing and follow-up procedures. With the introduction of cyclosporin A in the early 1980s, the effectiveness of immunosuppression dramatically improved. Hand transplantation was revisited and successfully performed in 1998 in France.⁵ Over time, the patient refused to adhere to the postoperative antirejection and physical therapies prescribed to him. His arm was amputated almost 3 years after surgery.⁵ To date, 25 hand transplants have been performed worldwide. All but two are viable. An additional hand amputation was performed on a patient that presented with severe skin inflammation who responded poorly to any treatment. The most successful of these transplants with longest follow-up was performed in Louisville, Kentucky (Fig. 2).⁶

Tendon Allotransplantation

Prior to performing the first successful hand transplant, each component of the CTA complex had been successfully transplanted, including tendon, nerve, bone, skin, and cartilage. Nonvascularized allotransplants of frozen or fresh tendons had previously been performed to replace lost/nonfunctional upper-extremity flexor tendons; however, the functional outcome of these allografts was unacceptable due to the reduced viability of the grafts and the disorganization of the flexor apparatus. In 1988, a French team directed by J. Guimberteau performed two allotransplantations of the digital flexor tendon apparatus collected from a living nonrelated donor and from a deceased donor.⁷ The digital flexor tendons were revascularized onto the recipient's ulnar vessels.⁸ Despite the low antigenicity of the tendon tissue, an immunosuppressive regimen was administered. The treatment protocol consisted of multiple doses of cyclosporin A (CyA; 7 mg/day) for a 6-month period. The grafts were accepted by both recipients. In addition, finger range of motion was considerably improved.

Nerve Allotransplantation

Nonvascularized nerve allografts are the most comprehensively examined reconstructive transplants. Improved

Figure 2 Matt Scott, the world's first successful hand transplant recipient, being evaluated.

knowledge of nerve allograft regeneration in animals contributed to the advancement of clinical studies in this field.⁹ During the early 1990s, a team directed by S. Mackinnon presented the outcomes of peripheral nerve allotransplantations on seven patients after upper- or lower-limb trauma.¹⁰ The immunosuppressive therapy consisted of azathioprine, prednisolone, and CyA for five patients. Tacrolimus (FK506) replaced CyA for the other two patients. The limb grafts were harvested from deceased donors and preserved at 5°C for 7 days prior to transplantation. This technique, though only possible in nonvascularized allografts, decreased the expression of major histocompatibility complexes (MHC) on the cell surface, thus decreasing the immunogenicity of the transplant. The immunosuppression was stopped 6 months after neuronal regeneration was evident distal to the allograft nerve, thereby avoiding any immunosuppressive drug-induced complications. The results from these transplants varied between groups. The allograft of one patient was rejected after 3 months. Four patients who received both auto- and allografts displayed some functional recovery. Two patients who received only allografts failed to recover any motor function but regained some sensory function. This series of transplants were not true CTA, as the nerve allografts served only as structural support for the recipient neuronal tissue to grow on. Though specific conditions in the nonvascularized grafts such as graft preservation and the withdrawal of immunosuppression are not applicable to other tissues, this clinical series was the first to show that the circumvention of problems associated with transplantations could not be performed simply through the use of autologous tissues.

Knee Allotransplantation

In 1994, a German team directed by G. Hofmann began a program of transplantation of vascularized bone tissue. Hofmann performed the first allogeneic vascularized transplantation of a fresh and perfused human knee joint.¹¹ Based on P. Chiron's model of the first vascularized femoral diaphysis allograft,¹² three femoral diaphyseal allotransplants and five entire knee joint allotransplants were performed.¹³ Immunosuppressive therapy consisted of CyA, azathioprine, antithymocyte globulin, and methylprednisolone for the first 3 days, which was subsequently reduced to only CyA and azathioprine. After 6 months, CyA alone was administered until complete bone consolidation. Although the immunosuppressive regime was quite strong, the lasting outcome was poor. Two-year to 5-year patient follow-up revealed that one of three femur allografts and three of five knee allografts were rejected and consequently removed and replaced with either a bone autograft or a prosthetic device.¹⁴ Complications were believed to be attributed to the combination of immunosuppressive

drugs and to inadequate means of monitoring for rejection.

Larynx Allotransplantation

One of the most remarkable CTA cases was the first laryngeal transplant performed by M. Strome in 1998.¹⁵ An aphonic 40-year-old man who suffered laryngeal trauma received a complete pharyngolaryngeal complex, which included the donor larynx, five tracheal rings, thyroid, and parathyroid glands (Fig. 3). The donor's superior laryngeal nerves and right recurrent laryngeal nerves were sutured to the corresponding recipient nerves. The immunosuppressive therapy consisted of

Figure 3 (A) Depiction of the anatomy of laryngeal allograft. (B) Dr. Marshall Strome with the world's first laryngeal transplant recipient.

induction with anti-CD3 monoclonal antibody, CyA, methylprednisolone, and mycophenolate mofetil (MMF). The patient received FK506, CyA, MMF, and prednisolone for maintenance therapy. After the transplant, the patient required a tracheostomy. One-and-a-half years later, the patient suffered a rejection episode that was corroborated by laryngeal biopsies. The event was successfully treated over a few days with increased steroid dosage. In spite of these problems, the transplantation was praised as a great achievement. At follow-up 2 years after the laryngeal transplant, the patient's voice with encompassed tone, quality, intensity, and flow was normalized.¹⁶ His taste and olfactory senses improved, and he was able to feed himself without aspirating.

IMMUNOSUPPRESSIVE DRUGS AND TREATMENT PROTOCOLS IN TRANSPLANTATION

Graft outcome and patient survival after organ transplantation were disappointing until the early 1970s when the development of new pharmacological drugs significantly improved graft survival.¹⁷ The 8-year living donor kidney transplant survival rate improved from 50% in the 1960s to 80% in the 1990s.¹⁸ The addition of CyA, a calcineurin inhibitor, to the transplant surgeon's armamentarium dramatically improved outcomes of heart, kidney, and liver transplants. Similarly, the introduction of another calcineurin inhibitor, FK506, in the early 1990s was associated with improved outcomes.¹⁹ Lubitz et al showed that administration of FK506 within 24 hours after heart transplantation alone could provide adequate immunosuppression in a majority of patients.²⁰ With the advent of additional immunosuppressive drugs in the late 1990s, such as the purine synthesis inhibitor MMF and sirolimus (rapamycin), originally developed as an antifungal agent, transplant surgeons had an arsenal of immunosuppressant drugs readily available. Currently, combination immunosuppressant therapy is being utilized in organ transplantation to control graft rejection. Clinically, no single immunosuppressive agent can completely prevent posttransplantation immunoreaction; however, the use of multiple agents with non-overlapping toxicity has dramatically improved outcomes. The combination of FK506/MMF treatment appears to be most advantageous when compared with either FK506/rapamycin or CyA/MMF treatments in cardiac transplant patients.²¹ Moreover, conversion of kidney transplant patients from FK506/rapamycin treatment to FK506/MMF treatment led to improved renal function.²² Currently, the most commonly used immunosuppressive treatment regimen in hand transplant patients is induction with antithymocyte globulin (ATG), FK506, MMF, and steroids. Most patients are maintained on a treatment protocol of FK506, MMF, and steroids.⁶ However, steroid-sparing approaches that

were initially developed in abdominal organ transplant recipients are now being tested.

CTA results in small-animal models were discouraging in the mid-1990s until the introduction of MMF. Graft and survival rates in rat hind-limb transplant models increased when MMF was combined with CyA²³ and when MMF was combined with FK506.²⁴ Recently, a new synthetic immunomodulatory drug, FTY720 (fingolimod), has been shown to inhibit lymphocyte movement out of the lymph nodes and Peyer's patches, thus sequestering lymphocytes from the allograft.²⁵ Though quite novel in its mechanism, FTY720 failed phase II trials and is no longer in clinical development.²⁶ Currently, a novel oral immunosuppressant under development has shown some promise: FK778 (malononitrilamide), a malononitrilamide, which suppresses T- and B-cell proliferation via inhibition of pyrimidine synthesis.²⁷ Yamamoto et al showed that treatment of recipient rats with FK778 was beneficial in preventing acute rejection. In addition, the condition of the graft was better when transplanted rats were treated with a combination of FK778 and FK506 compared with FK778 alone.²⁸ Although experimental immunosuppressive agents such as FK778 are promising, it is doubtful that these new agents will be obtainable before 2010.²⁹

Maintenance Immunosuppression

The primary problem confronting CTA is the requirement for recipients to be subjected to lifelong immunosuppressive therapies and the side effects associated with these drugs.¹ Research groups are currently attempting to remove or reduce maintenance immunosuppression that potentially leads to life-threatening complications (Table 1). The type of side effects associated with the use of each agent must be known to determine the associated risk for each CTA recipient. Calcineurin inhibitors (CyA, FK506), inhibitor of the mammalian target of rapamycin (mTor), antiproliferative agents (MMF), and corticosteroids (prednisolone) are classes of immunosuppressants currently used in transplantation. The side effects associated with current immunosuppressive drugs can be categorized into three groups: drug toxicity, opportunistic infections, and malignancies (Table 1).³⁰

Opportunistic infections in immunosuppressed organ recipients are a major cause of death after transplantation. They are generated by ubiquitous and often endogenous environmental organisms. The innate immune system, normal flora, and acquired immune mechanisms offer the necessary protection against opportunistic organisms in immunocompetent hosts.³¹ Almost 80% of organ recipients develop some form of infection.³² In the first month after transplantation, more than 95% of infections in transplant recipients are caused by the same microorganisms that cause perioperative infection in non-immunosuppressed patients. Between

Table 1 Complications of Immunosuppressive Drugs

Immunosuppressive Drug	Complication
Cyclosporine	Hypertension
	Hyperkalemia
	Nephrotoxicity
	Hirsutism
	Gingival hyperplasia
	Hepatotoxicity
	Hyperuricemia
	Hypertriglyceridemia
	Hypercholesterolemia
	Paresthesia
Tacrolimus	Malignant disease
	Nephrotoxicity
	Neurotoxicity
	Hyperglycemia
	Diabetes
	Paresthesia
	Psychosis
Sirolimus (rapamycin)	Encephalopathy
	Leukopenia
	Thrombocytopenia
	Hyperlipidemia
Mycophenolate mofetil	Gastrointestinal side effects
	Leukopenia
	Neutropenia
	Lymphoma
	Lymphoproliferative disease

Source: Adapted from Randhawa M. Update on immunosuppressive drugs. Drug Topics – The Online Newsmagazine for Pharmacists. 2001. Available at: <http://www.drugtopics.com/drugtopics/article/articleDetail.jsp?id=118767&searchString=update%20on%20immunosuppressive%20drugs>.

1 and 6 months after transplant, 90% of opportunistic infections in recipients are due to coinfecting viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human immunodeficiency virus (HIV). Almost 80% of patients who experience good transplantation results for ≥ 6 months are primarily at risk of community-acquired respiratory viruses.³³

Patients on chronic immunosuppression experience a significant increase in malignancy compared with non-immunosuppressed controls (Israel Penn International Transplant Tumor Registry data). Of the total number of de novo tumors from organ transplant recipients reported to the Cincinnati transplant tumor registry, ~40% were of skin and lip origin and 3.5% were carcinomas of the kidney.³⁴ With the introduction of novel immunosuppressant reagents, there has been no detectable change in the incidence of non-skin cancers thus far, but improved immunosuppression appears to increase the incidence of skin cancer. Otherwise successful, organ transplantations have failed as a result of the onset of cancer.³⁵ Because the immunosuppressive pro-

tolcols for hand transplant recipients is similar to those of renal transplant recipients, the incidence of malignancies is expected to be similar to that for solid organ transplant recipients.

In addition to immunosuppression-induced malignancies, nephrotoxicity is a serious side-effect of chronic immunosuppression (Table 1). Calcineurin inhibitors, FK506 and CyA, both have been shown to impair renal function.³⁶ Chronic kidney disease in non-renal transplant recipients ranges between 7% and 21%, depending on the type of organ transplanted. The 5-year risk of developing chronic kidney disease in heart and lung transplants is 15.8% and 10.9%, respectively. In addition, non-renal transplant recipients with chronic renal failure (CRF) have an increased mortality risk by a factor of 4.³⁷ Approximately 2 to 10% of orthotopic liver transplant recipients on a calcineurin-based immunosuppressive regime acquired end-stage renal disease (ESRD) 10 years after transplantation.³⁸ Hendawy et al showed that ~6 years after heart transplantation, calcineurin inhibitor toxicity caused CRF in 38.9% patients and the progression into ESRD. Moreover, 50% of patients with ESRD and 18.5% of patients with CRF died as a result.³⁹

Antigenicity of Composite Tissue Allotransplantation

CTA transplants are attached to recipient vasculature, which requires the grafting of skin, muscle, nerves, bone, and cartilage.⁴⁰ Each limb allograft tissue type has distinct immunologic elements such as MHC expression and immunogenicity. Transplanted skin and vasculature have been shown to elicit both cellular and humoral immune responses, whereas transplanted muscle, nerve, bone, and cartilage primarily elicit cellular responses.⁴¹ Many predicted that rejection would be difficult to control in such an array of complex tissues. Interestingly, Lee et al demonstrated that when limb allografts containing these different tissue types were transplanted, rejection was slower and the immune response generated was decreased.⁴⁰ This occurrence may have been the result of an overwhelmed immune system, enhancing antibodies, or activation of suppressor or regulatory T cells.

Chronic Rejection

Chronic rejection is the predominant cause of graft failure after organ transplantation and may pose a challenge for CTA as well. Acute rejection develops between 3 and 6 months after transplantation. Chronic rejection occurs slowly over months to years after transplantation and progresses throughout the life of the transplant. It is the major cause of mortality after lung and heart transplantation. The 1-, 3-, and 5-year survival rates after

Table 2 Estimates of the Incidence of Chronic Rejection

Allograft	Approximate Incidence of Obliterative Arteriopathy at 5 Years After Transplantation
Heart	25–60%
Heart-lung	8–20% (obliterative arteriopathy in heart) 50–60% (obliterative bronchiolitis in lung)
Kidney	40–50% (chronic allograft nephropathy)
Isolated lung	28%/45% (single/double lung) to 80%
Liver	4–6% in adults 8–12% in children
Pancreas alone	30–70% * (late graft loss)
Pancreas and kidney	20–40% *

*Estimates based on graft survival rates after 1 year, with the assumption that graft failures are due to chronic rejection. Adapted from: Demetris AJ, Duquesnoy RJ, Fung JJ, Murase N, Nalesnik M, Parmjeet R, Wu T, and Zeevi A. Pathophysiology of chronic Allograft Rejection. 2001, Available at: http://www.medscape.com/view_program/336_pnt.

lung transplantation are 75%, 58%, and 44%, respectively.⁴² The 5- and 10-year survival rates for heart transplantation are 75% and 51%, respectively.⁴³ Table 2 summarizes the incidence of chronic rejection in solid organ allografts. All organs undergo pathophysiologically similar changes (arterial intimal hypertrophy, interstitial fibrosis, atrophy), often resulting in organ failure. Chronic rejection may have great impact in CTA. Matt Scott, the world's first successful hand transplant recipient, showed no signs of chronic rejection at his 7-year check-up. Given that half of hand transplant recipients have experienced some form of acute rejection, one would expect these patients would develop chronic rejection and, hence, loss of function.

Graft-Versus-Host Disease

Because of its heavy lymphoid burden, CTA is believed to carry a risk of GVHD. When the migration of mature immunocompetent donor-specific T cells from the transplanted organ to the host tissue initiates an immune response against the recipient's body, the host may suffer graft-versus-host disease (GVHD). Cytotoxic CD8⁺ T cells and NK cells are the effector cells from the donor predominately responsible for the onset of GVHD.⁴⁴ Acute GVHD occurs within a few days (HLA-mismatched recipient) or can be delayed for up to 2 months after transplantation. The common target sites for GVHD include the skin, leading to an erythematous and maculopapular rash of the palms, the liver (leading to jaundice with conjugated hyperbilirubinemia), and the gastrointestinal tract (which can lead to diarrhea, abdominal cramps, distention, and bleeding).⁴⁵ Chronic GVHD presents after 3 months and is defined as GVHD syndrome that may develop from an extension of acute GVHD or after a disease-free period. The incidence of chronic GVHD ranges from 30 to 60% in marrow or hematopoietic cell transplant recipients and is

correlated with the degree of donor-recipient HLA disparity.⁴⁶ Though any tissue rich in donor lymphocytes has the potential to cause GVHD, the incidence of GVHD correlates with the specific tissue transplanted.²⁴ Lymph nodes contain the highest number of T lymphocytes compared with other tissues and thus have the potential to give rise to GVHD.³ Likewise, transplanted hand or arm bone marrow compartments are rich in donor T cells and also have the potential to cause GVHD. To decrease the risk for GVHD, some groups have irradiated the donor limb prior to transplantation.⁴⁷ However, no GVHD has been observed clinically in recipients of nonirradiated limbs to date.

STRATEGIES FOR TOLERANCE INDUCTION

The ideal immunosuppressant is one that allows for the reprogramming of the recipient's immune system to induce donor-specific tolerance to the graft. Though newer pharmacological agents have greatly benefited current successes in solid organ transplantation and also CTA, the fundamental goal is to achieve tolerance without the use of immunosuppressive drugs. Many groups are currently investigating various strategies to induce tolerance to CTA, including T-cell depletion, the administration of tolerogenic donor dendritic cells, T-cell costimulatory blockade, gene targeting, and mixed chimerism. The requirement for chronic immunosuppression and occurrence of chronic rejection may be eliminated if tolerance is induced.

T-Cell Depletion

Conventional "one-size-fits-all" immunosuppression typically consists of a calcineurin inhibitor (cyclosporine or FK506), MMF, Imuran (azathioprine; Prometheus Laboratories, Inc., San Diego, CA), or rapamycin, and steroids. More recently, steroid-sparing approaches have proved successful, and immunosuppression is being more individually tailored. T-cell depletion (lymphodepletion) before allotransplantation with T-cell repopulation post-transplantation has been shown to induce graft acceptance with reduced immunosuppression in some animal models as well as in humans. Lymphodepletion induction therapy has allowed steroid-sparing protocols to become the preferred therapy in solid organ transplantation. T-cell depletion can be achieved through the use of Campath-1H (alemtuzumab; Millenium Pharmaceuticals, Cambridge, MA),⁴⁸ Orthoclone (OKT3), or Thymoglobulin (anti-thymocyte globulin [ATG]; Fresenius, Cambridge, MA).²⁶ Campath-1H is a monoclonal antibody that reacts against CD52 surface antigen expressed on T and B cells. The beneficial use of Campath-1H has been seen in human CTA. Campath-1H treatment prevented allograft rejection when administered with FK506 monotherapy.⁴⁹ Kaufman et al

demonstrated that a combination of Campath-1H and antithymocyte globulin was effective in preventing acute rejection in simultaneous pancreas-kidney transplants patients.⁵⁰ Moreover, Campath-1H induction, followed by maintenance therapy with FK506 and rapamycin, was shown to be safe and effective in long-term kidney⁵¹ and kidney/pancreas organ recipients.⁵⁰ More recently, similar success has been observed in cardiac transplant recipients.⁵²

In Vitro Manipulated Donor Dendritic Cells

Dendritic cells (DCs), the most potent of professional antigen-presenting cells, play a central role in regulating the generation and control of innate and acquired immunity.⁵³ Several DC subsets have now been identified, each with specific functions. Induction of immunity or tolerance is dependent on the dendritic cell state of functional maturation. Immature DCs can induce transplant tolerance via immune deviation, T-cell anergy/apoptosis, or regulatory T-cell generation.⁴⁵ Lutz et al demonstrated that recipient mice transplanted with immature DCs derived from donor bone marrow 7 days prior to transplantation had significantly prolonged cardiac allograft survival.⁵⁴ Fugier-Vivier et al showed that CD8⁺/TCR⁻ facilitating cells (FCs), the majority of which express a precursor plasmacytoid DC phenotype, significantly improved engraftment of hematopoietic stem cells (HSCs) and induced donor-specific tolerance to skin allografts.⁴⁶ FCs have recently been shown to convert CD4⁺/CD25⁻ T cells to Foxp3⁺/CD4⁺/CD25⁺ T_{reg} in the presence of

cytosine-phosphate guanosine (CpG).⁵⁵ In addition, Yoshimura et al showed that nuclear factor- κ B gene transcription plays an important role in DC maturation and function.⁵⁶ Protocols to immunologically manipulate transplant recipients with tolerogenic cells are currently being tested in the clinic, including those using CD8⁺/TCR⁻ FC.

T-Cell Costimulatory Blockade

The most promising approach to induce peripheral tolerance is costimulatory blockade. To generate an immune response, antigen recognition (signal 1) by MHC complexes of APCs must be associated with further costimulatory signals (signal 2). T-cell receptor engagement alone with antigen is not enough to elicit a T-cell response and therefore requires a second signal such as membrane expressed costimulatory molecules. Costimulatory blockade refers to the blocking of signal 2 after transplantation, which results in rendering the recipient unresponsive to donor antigen. By blocking signal 2 in the presence of signal 1, alloreactive T and B cells become anergic or apoptotic. Many costimulatory molecules, including CD28, B7, CD40, and CD154 (CD40L), have been defined⁵⁷ (Fig. 4). These molecules control alloimmune responses by regulating lymphocyte activation, proliferation and differentiation. CD28 is expressed on resting and activated T cells. B7-1 and 2 are both ligands for CD28 that are expressed on APCs. B7 and CD28 interactions facilitate T-cell activation, which results in T cell receptor (TCR) signaling, IL-2 production, and T-cell proliferation.⁵⁸ CD154 binding

Figure 4 Association of CD4⁺ helper T cells with APCs requires the interaction of multiple T-cell membrane proteins with their respective receptors on the target APC. (Adapted from: Abbas AK and Lichtman AH. Cellular and Molecular Immunology. 5th ed. Amsterdam: Elsevier; 2005:114, Fig. 6–8.)

to CD40 has been shown to upregulate cell adhesion molecules in endothelial cells required for the migration to inflammatory sites and is important in the generation of humoral responses.⁵⁹ Blocking secondary signals with monoclonal antibodies results in inhibition of T-cell activation and sustained downregulation of immune responses, thereby prolonging graft acceptance. Elster et al showed that treatment of rhesus monkeys with anti-CD154 delays acute skin allograft rejection.⁶⁰ Although a promising therapy, anti-CD154 may have limited use in CTA because of prothrombotic properties associated with its use in non-human primates.⁶¹ In other studies, Haspot et al demonstrated that blockade of the CD28/B7 pathway induced kidney allograft tolerance in a rat model.⁶² All studies using short-term cytotoxic T-lymphocyte antigen 4 (CTLA-4) immunoglobulin therapy to block CD28/B7 costimulation have shown increased allograft survival.⁶³ In addition, combined blockade of CD40/CD154 and B7/CD28 pathways can effectively result in long-term acceptance of skin and cardiac allografts in small animals and non-human primates.^{48,64} The promise of this approach in the clinic is just now being evaluated. One major unanswered question is whether chronic rejection would be prevented, as throughout life humans likely continue to produce potentially alloreactive T and B cells.

Gene Targeting of Transplanted Organs

Gene therapy may offer several methods for achieving induction of tolerance in mammals. Although induction of graft tolerance by gene targeting has experienced some success in rodent models, tolerance in large animals and human models has not yet been attained. A major limitation of gene therapy has been the generation of an immune response by the recipient to the vectors used to insert the gene of interest. Bagley et al demonstrated that transferring donor-specific genes such as MHC class I and II into the recipient via

donor-specific transfusion induces a state of micro-chimerism where the recipient's bone marrow expresses donor protein. As such, the newly formed donor-specific MHC-expressing cells are protected from immune destruction.⁶⁵ Other groups are currently using retroviral vectors as a method of gene transfer. Many advances have been made in the past 5 years in the development of improved viral vectors. Lentivirus, adeno-associated viruses (AAV), adenovirus, and HSV-1 derived vectors do not contain wild-type viral genes. Consequently, the only antigens recognized by the immune system are the viral envelope and viral capsid proteins. Once the vector genome enters the nucleus of the cell, the host can then express the therapeutic transgene long-term while remaining undetected by the immune system. Although patients can potentially mount an immune response against the input virions, transient immunosuppression during the vector uncoating phase could be sufficient in blocking this response.⁶⁶

Mixed Chimerism

Deletional, or central, tolerance is believed by some to be the most robust form of donor-specific tolerance, as donor-reactive cells are actively deleted from the repertoire. One of the most successful strategies and best-studied procedures to achieve central tolerance is through bone marrow transplantation (BMT), which results in HSC chimerism (Fig. 5). In 1984, Ildstad et al reported in *Nature* that mixed chimerism induced tolerance to skin allografts and xenografts. Recipients with as low as 1% donor MHC class I chimerism were tolerant to skin, heart, islet, lung, and endocrine grafts.⁶⁷⁻⁷⁰ This finding was important because it opened the door to the development of reduced-intensity conditioning strategies to minimize the risk associated with the procedure.⁷¹ Li et al found that when 200 cGy total body irradiation (TBI) was combined with immunosuppression of the

Figure 5 Mixed hematopoietic chimerism for induction of donor-specific tolerance.

recipient, mixed chimerism could be established with as little as 200 cGy TBI. This approach has been safely translated to the clinic, with more than 400 procedures performed worldwide.⁷²

Although tolerance is readily achieved in rodent models, it was previously questioned whether similar success would occur in humans. Chimerism-induced allograft tolerance was first reported in 1981 in a patient who required bone marrow from an HLA-identical sibling because of acute myelomonocytic leukemia. The patient gradually lost renal function and subsequently underwent renal allotransplantation. The same sibling who donated the bone marrow donated the kidney, which resulted in allograft acceptance without immunosuppression.⁷³ All patients who have undergone similar procedures have accepted their kidneys without long-term immunosuppression. Research is currently being performed to develop a nontoxic tolerance induction regime that can be used routinely for organ transplantations. Wekerle et al demonstrated in a mouse model that when bone marrow is administered in combination with costimulatory inhibitors, anti-CD154 and CTLA-4 immunoglobulin, both important in preventing complete T-cell activation, allogeneic bone marrow engraftment was achieved without cytoreduction or T-cell depletion of the recipient.⁷⁴

Skin has been considered a highly antigenic challenge for testing tolerance. It was therefore debated whether tolerance could be induced to CTA. Prabhune et al demonstrated that transplantation of donor bone marrow cells into conditioned recipients after limb transplantation can induce mixed chimerism, tolerance, and allograft survival in a rat model.⁷⁵ Also in a rat model, Demir et al showed that donor-specific chimerism and functional tolerance can be induced in hemifacial allograft transplants using a CyA monotherapy protocol.⁷⁶ Foster et al demonstrated that CD28 blockade and mixed chimerism inhibits both *in vitro* and *in vivo* expansion of the T-cell repertoire and prevents acute and chronic rejection in rat hind-limb allografts.⁷⁷ In addition, Li et al showed in rat model that induction therapy with CTLA-4-Ig, FK506, and anti-lymphocyte serum (ALS) results in durable mixed chimerism at lower TBI doses (300 to 400 cGy) and no detectable GVHD.⁷⁸ Moreover, in a similar rat induction protocol, FK506 and ALS treatment with TBI dose of 500 cGy results in mixed chimerism and acceptance of a nonfunctional hind-limb CTA up to 150 days (Fig. 6) (Adamson LA, Huang WC, Breidenbach WC, et al. A modified model of hindlimb osteomyocutaneous flap for the study of tolerance to composite tissue allografts. *Microsurgery* 2007 Sep 14; [E pub ahead of print]). Though some groups attempted to minimize the toxic effects of higher induction dose therapies, these attempts were not very successful as long-term allograft survival could not be achieved.⁷⁹ Siemionow et al demonstrated that mainte-

Figure 6 Chimeras accept CTA.

nance of donor-specific chimerism and operational tolerance could be achieved in 100% of hemifacial allograft recipients from semi-allogeneic and fully MHC mismatched donors through a high induction dose of CyA and low maintenance doses of CyA monotherapy.⁸⁰ In addition, Siemionow et al have introduced a novel procedure for the transplantation of donor-derived CD90⁺ hematopoietic stem and progenitor cells. Intraosseous injection of donor-derived stem cells abolishes the homing process and leads to increased competition for vacant niches thereby, establishing donor-specific chimerism and increasing limb allograft survival.⁸¹

FUTURE DIRECTION FOR COMPOSITE TISSUE ALLOTRANSPLANTATION

Continued advances in research and outcomes in clinical CTA have convincingly shown that it is a viable therapeutic option and have ensured its sustained placement in the future of transplant surgery. Although solid organ transplants are viewed in most cases as lifesaving, CTA affords the patient a significant improvement in quality of life, especially when both limbs have been lost. In addition, CTA tolerance is hypothesized to be more challenging to attain than organ tolerance because of the immunologic complexity of the CTA graft. The ability to restore anatomic and cosmetic integrity with functionality has to be balanced with long-term and potentially toxic immunosuppression and morbidity due

to the surgical procedure. Moreover, to achieve restoration of functionality, acute rejection should be prevented and the potential for chronic rejection diminished. Thus far, the ability to restore functional integrity while sustaining well-tolerated immunosuppressive protocols has been demonstrated in 25 patients who received hand transplants worldwide. CTA can be utilized by reconstructive surgeons to significantly improve the lives of disfigured or crippled individuals. As it becomes widely applied, the millions who would benefit will be challenged by availability of appropriate donors. In any case, ongoing clinical and scientific research continues to discover and develop new immunosuppressive therapies and lasting patient care management. Moreover, the long-term promise of stem cell-based therapies to generate new tissue may take CTA to its next level.

ACKNOWLEDGMENTS

The authors thank Dr. Dina Rahhal and Larry Adamson for review of the manuscript and helpful comments, Carolyn DeLautre for manuscript preparation, and the staff of the animal facility for outstanding animal care. This research was supported in part by the Department of Defense, Office of Naval Research N00014-06-1-0189; the Department of Defense, Office of Army Research 06075002; The Commonwealth of Kentucky Research Challenge Trust Fund; The Jewish Hospital Foundation; and the University of Louisville Hospital.

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