10-1-2002

The spectrum of complications of immunosuppression: Is the time right for hand transplantation?

Michael J. Brenner
Washington University School of Medicine in St. Louis

Thomas H. Tung
Washington University School of Medicine in St. Louis

John N. Jensen
Washington University School of Medicine in St. Louis

Susan E. Mackinnon
Washington University School of Medicine in St. Louis

Current Concepts Review

The Spectrum of Complications of Immunosuppression: Is the Time Right for Hand Transplantation?

By Michael J. Brenner, MD, Thomas H. Tung, MD, John N. Jensen, MD, and Susan E. Mackinnon, MD

Investigation performed at the Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, St. Louis, Missouri

- Life-threatening complications of long-term immunosuppression include malignancy, infection, and metabolic disorders such as renal failure and diabetes.
- Up to three-fourths or more of patients on chronic immunosuppressive medications experience an infectious complication.
- The hand transplants to date have had multiple episodes of acute rejection.
- The frequency and timing of episodes of acute rejection, even if the episodes are easily treated, are predictive of chronic allograft dysfunction and failure.
- Chronic allograft rejection is not effectively treated with current immunosuppressive medications, and it has become a primary cause of long-term allograft failure.

The introduction of cyclosporine A in the early 1980s revolutionized solid organ transplantation. There were marked improvements not only in preserving graft function, but also in prolonging patient survival following solid organ transplantation[1]. As the viability of transplanted organs improved, the indications for clinical transplantation expanded to include not only acutely life-threatening conditions, but also more chronic conditions that adversely affected longevity and quality of life.

Composite tissue allotransplantation involves the transplantation of nonvital tissues for reconstruction of deficits following trauma or tumor resection. If successful, composite tissue allotransplantation would facilitate the recovery of lost function through grafting of complex, disparate tissue types. However, composite tissue allotransplantation also poses unique challenges. Solid organs, such as the kidneys, may be more tolerant of rejection episodes than are composite tissues, for which the risk of rejection of one or more components is high. Parenchymal organs may have a tremendous functional reserve so that a substantial amount of tissue can be lost before organ function is compromised, but composite tissue allografts tend to have complex architecture with limited built-in redundancy. Thus, while the majority of kidney function may be lost before blood urea nitrogen or creatinine levels are elevated, comparable loss of the function of composite tissue allotransplants may have more profound consequences. In the hand, for example, many fine anatomical components must work together to achieve precise motor movements. Rejection episodes that only impair nerve regeneration or result in loss of muscle could result in irreversible loss of function.

Despite these obstacles, enthusiasm for composite tissue allotransplantation has grown. In 1991, a composite tissue transplantation workshop was held in Seattle, Washington, under the sponsorship of the Rehabilitation Research and Developmental Service of the Department of Veteran Affairs[2]. Literature and scientific progress in this area were reviewed extensively by transplantation biologists and clinical transplantation surgeons. Ultimately, the participants at this conference concluded that more research and clinical progress were necessary before clinical trials could be undertaken. In 1997, the first International Symposium on Composite Tissue Allotransplantation was held at the Jewish Hospital in Louisville, Kentucky. The scientific literature was again reviewed, and the controversy of clinical application was extensively debated. Llull suggested that a moratorium be placed on transplantation of the hand[3], but others thought that "the time [was] ripe to proceed with the first human cadaveric hand transplantation."[4,5] Despite opposition from the majority of the hand surgery community, the first hand transplants were performed in Lyon, France, and Louisville, Kentucky, in 1998, and other transplants in China, including bilateral transplants, have followed[6].

TABLE I Complications of Immunosuppressive Drugs

Toxicity
Malignant disease
Infection
Nephrotoxicity
Hypertension
Hyperlipidemia
Neurotoxicity
Diabetes
Osteoporosis
Gingival hyperplasia
Acne
Hirsutism
Alopecia
Gastrointestinal toxicity
Heme toxicity

Since 1998, hand transplantation has remained a divisive subject in the hand surgery community[7-12]. The controversy centers on the need for indefinite high-dose immunosuppression for allograft survival. While many experts identify composite tissue allotransplantation as an important long-term goal of hand and reconstructive surgery, there is debate surrounding both the hand transplants already performed and the wisdom of proceeding with future clinical trials. The controversy initially focused on whether the performance of the clinical hand transplantations had been premature as demonstrated by existing experimental data that predicted early graft failure[7,13-15]. With the success of several clinical hand transplants that have now lasted for more than two years, the debate has shifted to potential side effects of long-term immunosuppression and the likelihood and timing of chronic rejection and graft failure. The literature on solid organ transplantation provides insight into these issues.

The discussion that follows will address the following questions: What are the possible benefits of hand transplantation? What are the associated risks? In particular, what are the sequelae of chronic immunosuppression? Can acute rejection episodes predict chronic rejection and graft failure? What criteria can be used to determine whether there have been sufficient preliminary studies of composite tissue allotransplantation to justify its experimental use in patients?

Benefits of Composite Tissue Allotransplantation

Much of the argument for composite tissue allotransplantation is based on the success of reconstruction of major defects of the extremities with autologous tissue[16]. The most relevant literature concerns the replantation of amputated digits and limbs and the free transfer of analogous tissue components (for example, toe-to-thumb transfers)[17]. While autologous reconstruction is a well-accepted option for the treatment of anatomical functional deficiencies of the hand, an unfavorable amputation site or a lack of suitable tissue for replantation may render this option impractical or impossible. In this setting, allotransplantation is likely to offer the best prospect for functional recovery and restoration of body image. Composite tissue allotransplantation could allow reconstruction of several specialized tissues other than the extremities, such as the larynx[18,19], the tongue[20], and potentially other maxillofacial components.

There are several theoretical benefits of hand transplantation. The anatomy of the hand is complex, and there are few satisfactory alternatives to reconstruction with autologous tissue when replantation is not possible. With transplantation of a cadaveric hand, the desired tissue can be procured without donor-site morbidity and can be adapted to the needs of the host environment. For example, an allograft can be fashioned to make productive use of the damaged nerves, vessels, tendons, and joints proximal to the amputation level. Although imperfect, a hand allograft would more closely replace the appearance, anatomy, and function of a native hand than would any other available reconstruction. In addition, from a psychological standpoint, a hand transplant might counter the devastating effects on body image that can occur with loss of a hand.

Risks of Immunosuppression

The possible benefits of composite tissue allotransplantation, particularly hand transplantation, must be weighed carefully against the risks of indefinite immunosuppression. On the basis of clinical experience with solid organ transplantation, it can be anticipated that the major risks include the possibility of graft-versus-host disease and the morbidity resulting from episodes of graft rejection[6]. To this list should be added certain considerations that are unique to composite tissue allotransplantation. For example, what are the psychological and emotional ramifications of having a hand transplant that deteriorates in appearance or function, or both? The recipient of the world's first "successful" hand transplant, which was recently amputated because of rejection, stated that he had no normal sensation in the hand and experienced pain and burning sensations. Signs of rejection are grossly evident as skin erythema progresses to epidermolysis and eschar formation. The patient became emotionally detached from the allograft and "realized that it wasn't my hand after all."[12,21] Perhaps a more important question is: Should a hand transplant fail, what does it mean to a patient to lose a hand not once, but twice?

The complications of lifelong immunosuppression are well defined and some are potentially life-threatening. Nonspecific immunosuppression is the only established clinical method currently available for sustaining an allotransplant. Because composite tissue allotransplantation involves multiple heterogeneous tissue types, some of which are highly antigenic, not

TABLE II Infections in Transplant Recipients[27]

Infection	Prevalence of Infection (%)			
	Liver Transplant	Kidney Transplant	Heart Transplant	Lung Transplant
Bacterial	33-68	47	21-30	35-66
Cytomegalovirus	22-29	8-32	9-35	53-75
Herpes simplex virus	3-14	53	1-42	10-18
Varicella zoster virus	5-10	4-12	1-12	8-15
Candida	1-26	2	1-5	10-16
Mycelial fungi	2-4	1-2	3-6	3-19
Pneumocystis carinii pneumonia	4-11	5-10	1-8	15

only must immunosuppression be chronic, but it also must be maintained at a high level. The outcomes associated with such a regimen are not fully known, but a large body of literature addresses the prevalence of complications observed during treatment with immunosuppressive drugs (Table I).

When the complications are considered collectively, lifelong immunosuppression can be throught of as a chronic disease characterized by its own set of risks. Much as chronic hypertension increases one's risk of stroke or myocardial infarction, prolonged immunosuppression increases the risk of infection, fracture, neoplasia, drug toxicity, and metabolic derangement[13,18-39]. Unlike hypertension, however, chronic immunosuppression is usually symptomatic. Patients on immunosuppressive regimens frequently experience undesirable changes in appearance or behavior[22,23]. Patients may also have nausea, diarrhea, or a variety of other side effects[24,25]. The morbidity of this new disease profoundly affects the quality of life, alters the risk profile of composite tissue allotransplantation, and therefore must figure prominently in the balance of risks versus benefits when determining whether to initiate treatment.

Infection

The majority of patients who have had a transplant have an infection as a result of immunosuppression. In one study, a major infection developed in up to 88% (107) of 122 patients who had undergone kidney, heart, or liver transplantation[26]. Infections account for a major part of postoperative morbidity after solid organ transplants (Table II). The etiology may be bacterial, viral (cytomegalovirus, herpes simplex virus, or varicella zoster virus) or fungal, and the prevalence of these infections varies with the type of transplant and the degree of immunosuppression. Bacterial infections are the most common and have been reported in 21% to 68% of transplant recipients, and cytomegalovirus and herpes simplex virus may occur in up to 50% (or more) of patients[27,28]. In the early postoperative period, infections are surgical complications and include wound infections; pneumonia; urinary tract infection; *Clostridium difficile* colitis; infection at the site of a drain, an indwelling central catheter, or another type of catheter; and a hematoma or lymphocele surrounding the graft[26]. Opportunistic pathogens that are characteristic of patients who have had a transplant include cytomegalovirus, *Pneumocystis carinii,* Aspergillus species, Nocardia species, *Listeria monocytogenes,* and Toxoplasma, and they usually appear after the first month[26]. Pathogens already present in the transplant recipient may be reactivated by immunosuppression; these include *Mycobacterium tuberculosis, Histoplasma capsulatum, Coccidioides immitis,* and occult bacterial or viral infections. Chronic or latent infections that may be transmitted through the allograft include human immuno-

TABLE III Hypertension and Renal Dysfunction in Transplant Recipients

	Prevalence of Complication (%)				
	Liver Transplant (at 1-5 Yr)	Heart Transplant (at 1 Yr)	Heart Transplant (at 5 Yr)	Lung Transplant (at 1 Yr)	Lung Transplant (at 5 Yr)
Hypertension	30-90[34]	68[42]	68[42]	48[42]	63[42]
Renal dysfunction	65-80	13-52	14.0[42]	12.6[42]	17.6[42]
Creatinine >5 mg/dL (>442 μmol/L)	4[34]	7.8[42]	8.6[42]	8.00[42]	14.4[42]
Dialysis	2[34]	1.2-4.49[30,38,42]	1.9[42]-10[36]	1.60[42]	3.20[42]-10[36]
Renal Transplant	N/A	0.1[42]	0.4[42]	0.00[42]	0.7[42]

TABLE IV Fractures in Transplant Recipients[39]

	Liver Transplant	Kidney Transplant	Heart Transplant
Time between transplant and fracture* *(mo)*	19.7 ± 14.1	15.7 ± 9.6	30.2 ± 39.4
Prevalence of fracture *(%)*			
Men	11 (3/27)	4 (11/261)	12 (6/51)
All women	9 (2/22)	13 (22/171)	30 (3/10)
Postmenopausal women	100 (2/2)	45 (10/22)	100 (3/3)

*Mean and standard deviation.

deficiency virus, hepatitis B and C, and fungal or mycobacterial infection[27].

Hypertension and Nephrotoxicity

The neurohormonal mechanisms that lead to hypertension and nephrotoxicity following transplantation are thought to be similar. Factors that contribute to hypertension include altered renal vascular reactivity and vasoconstriction, increased sympathetic tone, and sodium retention[29,30]. The development of hypertension after cardiac or liver transplantation has been reported in 60% to 90% of patients treated with cyclosporine A, with prevalences of fifty-two of eight-five patients after three months and thirteen of fifteen after three years in one study[31], and in 30% to 60% of patients treated with tacrolimus (FK506), with prevalences of eighteen of twenty-eight patients after two years[32] and 327 of 710 after five years[33,34]. The nephrotoxicity of immunosuppressants has also been well studied and is potentially the most serious side effect. Tacrolimus and cyclosporine A have both been associated with impairment of renal function. Table III shows the prevalence of renal failure in heart and lung transplant recipients. (Data on liver and renal transplant recipients are not included to avoid confounding with hepatorenal syndrome and transplant dysfunction, respectively.) The rate of renal dysfunction as defined by a serum creatinine level of >2 mg/dL (>176.8 μmol/L) has been reported to be as high as 52% (ninety-seven of 187) only two years after heart transplantation in patients being treated with cyclosporine A[35]. Progression to end-stage renal failure may occur within only five years postoperatively in up to 10% of patients (as determined with actuarial analysis of 200 patients) treated with chronic cyclosporine-A therapy after heart and lung transplantation[36,37]. The mechanisms of nephrotoxicity induced by cyclosporine A and tacrolimus are thought to be identical. In the acute phase, renal blood flow is reduced as a result of afferent arteriolar vasoconstriction secondary to sympathetic nerve stimulation, a relative increase in vasoconstricting prostaglandins (especially thromboxane A2), and release of endothelin (a powerful renal vasoconstrictor). Chronically, there is cumulative tubulointerstitial damage and fibrosis associated with arteriopathy[38]. In a review of the cases of forty-nine children followed for a mean of only twenty-nine months after a heart transplant, renal toxicity (defined with use of the same criterion as described above) was noted in 37% (fourteen) of thirty-eight patients taking tacrolimus as the initial primary agent and in ten of eleven patients who had initially been treated with cyclosporine A and then switched to tacrolimus because of persistent side effects or rejection[38].

Metabolic Disorders

The metabolic derangements associated with chronic immunosuppression take a variety of forms. Among the best studied are the increased risk of fracture due to loss of bone density[39,40] (Table IV) and the increased prevalence of hyperlipidemia and diabetes[30,34,41-45] (Table V). The specific abnormalities vary depending on the immunosuppressive regimen, with cyclosporine A, tacrolimus, and steroids being the most commonly implicated agents. In a review of 600 patients who underwent abdominal organ and heart transplantation, the prevalence of fractures was increased by as much as 9% in men and 25% in women and the prevalence was as high as 100% (five of five) in postmenopausal women[39]. Overall, the relative risk, compared with National Health Interview Survey data on fracture prevalence in the United States population, was thirteen times higher in men forty-five to sixty-four years of age and eighteen and thirty-four times higher in women twenty-five to forty-four years of age and those forty-five to sixty-four years of age,

TABLE V Metabolic Complications in Transplant Recipients

	Prevalence of Complication *(%)*				
	Liver Transplant (at 1-5 Yr)	Heart Transplant (at 1 Yr)	Heart Transplant (at 5 Yr)	Lung Transplant (at 1 Yr)	Lung Transplant (at 5 Yr)
Hyperlipidemia	20-75[34]	40.6[42]	43.8[42]	12.5[42]	20.5[42]
Diabetes	4-20[43]	20.8[42]	8[30]-16.2[42]	16.5[42]	16.1[42]

TABLE VI Cancer in Transplant Recipients

	Prevalence of Cancer *(%)*					
	Liver Transplant (Overall)	Kidney Transplant (Overall)	Heart Transplant (At 1 Yr)	Heart Transplant (at 5-10 Yr)	Lung Transplant (at 1 Yr)	Lung Transplant (at 5 Yr)
Post-Transplant lymphoproliferative disorder	1.7-13[34,46,50,93]	1.0[50,54,93,94]	1.0-13[42,50,54,93,95]	1.16[30,42]	1.8-20[42,47,50,54,93]	1[42]
Skin	2.2[97]	2-65.3[94,96,97]	1.3[42]	3-43[42,52,94,96,97]	0.6[42]	3.2[42]
Other	5[98]	3[94]	1.2[42]	3.2[42]	1.4[42]	1.6[42]
Total	12[98]	2-20[94]	3.5[42]	8.8[42]	4.4[42]	5.8[42]

respectively[39]. The prevalence of hyperlipidemia can be as high as 74% (146 of 198) after heart or liver transplantation[34,41]. The prevalence may be somewhat higher with cyclosporine A (74%; 146 of 198 patients) than with tacrolimus (51%; fifty-three of 103 patients), but it can be very high with either[34]. The prevalence of diabetes mellitus is reported to be as high as 20% one year after heart transplantation and 16.5% one year after lung transplantation (as determined on the basis of The Registry of the International Society for Heart and Lung Transplantation of 38,943 heart transplant and 2855 heart-lung transplant recipients)[42] and 29% after renal transplantation[44]. The mechanisms of these metabolic derangements have been reviewed in detail elsewhere[30,34,42,44,45].

Malignant Lesions

Recipients of organ transplants are at substantially increased risk for the development of cancer. The relative risk of certain types of malignant disease has been estimated to be up to 400 times that in the general population. The prevalences of different malignant lesions, organized by type of transplant, are shown in Table VI. The most common malignant lesions seen in transplant recipients are skin cancers, which account for 37% of post-transplant tumors (4305 of 11,483)[46]. The prevalence varies with the type of transplant, geographical location, and sun exposure. The prevalence of nonmelanoma skin cancers developing within twenty years after liver transplantation is 30% in temperate climates such as the Netherlands, but it rises to 70% (as determined with a life-table analysis of 1098 patients) in more equatorial locations such as Australia[47,48]. Similarly, the prevalence twenty years after renal allografting was reported to be 40% (as determined with a life-table analysis of 764 patients) in a Dutch study[49], but the prevalence twenty-four years after renal allografting was 66% (as determined from the Australia and New Zealand Combined Dialysis and Transplant Registry of 6596 patients) in Australia[50,51]. Skin cancer was found to occur in 43% of patients (cumulative prevalence in 455 patients) ten years after cardiac transplantation in Australia[52,53].

Post-transplant lymphoproliferative disorders are the second most common malignant diseases after transplantation, but they are more troubling as the mortality rate is 50% to 80%[54]. The prevalence of these disorders is lower (1%) after renal transplantation but has been reported to be as high as 20% in recipients of nonrenal allografts[50]. The prevalence after lung transplantation in children has been reported to be as high as 13% (sixteen of 128 patients), and the prevalence increases to 21% (thirteen of sixty-one) in patients with cystic fibrosis, a common indication for lung transplantation[55]. The prevalence has been found to be as high as 32% (thirteen of forty-one patients) after intestinal transplantation[54,56]. The increased prevalence of cancer in patients treated with immunosuppression is thought to result from impaired immune surveillance of aberrant cells. This weakening of the body's defenses allows malignant cells that would normally be destroyed in an immunocompetent individual to survive and multiply in an immunosuppressed host. Withdrawal of immunosuppression often enables the body to control the progression of lymphoproliferative neoplasia but increases the risk of graft rejection.

Chronic Allograft Dysfunction and Rejection

The frequency and timing of acute rejections predict chronic rejection and allograft failure. Chronic rejection is the most prevalent cause of long-term failure of allograft organ transplantation, and its prevalence has remained unchanged despite two decades of progress in immunosuppressive therapy and perioperative care[57]. Chronic rejection with loss of the allograft is seen in 52% of patients (thirteen of twenty-five) by five years after cardiac and lung transplants[42,58] and in >50% (as determined on the basis of multicenter data from the United Network for Organ Sharing [UNOS] and Eurotransplant) seven to eight years after renal allograft transplantation[59,60]. The literature on renal transplantation indicates that the risk of chronic rejection increases threefold with one acute rejection episode, twelvefold with two or more acute episodes, and twenty-six-fold with late rejection episodes (those occurring more than eight weeks after transplantation)[61]. Experience with the first hand transplants performed in Lyon, France, and Louisville, Kentucky, suggests that preventing chronic rejection of composite tissue allotransplants will not be easy. Acute rejection episodes were noted at eight, fifty-five, and seventy-four weeks in Lyon and at four to six, eighteen to twenty, and seventy-seven weeks in Louisville[6]. These frequent and late acute rejection episodes suggest a substantial risk of chronic allograft rejection.

TABLE VII Scheme for Classifying Different Types of Transplant Surgery

Surgical Indications	Clinical Transplants
Life-threatening disease	Heart, lung, liver
Chronic debilitating disease	Kidney, pancreas
Functional deficit without active disease	Peripheral nerve*, limb/composite tissue, traumatic vocal cord injury[18]

*Nerve allotransplantation requires temporary rather than indefinite immunosuppression.

While the principal risk of hand transplantation results from immunosuppression, the principal obstacle to functional recovery is rejection. Because of the risk of rejection, the results of hand transplantation may be poorer than those of hand replantation. Function must be restored for limb transplantation to be considered a success[62]. The factors limiting recovery of function are impaired nerve regeneration and loss of motor units and sensory receptors. Composite tissue allotransplantation involves transplantation of composite tissues containing highly antigenic properties. Nerve regeneration could theoretically be impaired in the setting of a large antigen load. In primate studies conducted in our laboratory, nerve allografts treated with anti-CD40 ligand, an agent that blocks the costimulatory pathway between T-cells and antigen-presenting cells, showed robust regeneration. However, addition of a skin graft led to rejection in the same model, suggesting that one antigenic tissue component may predispose other tissue components to rejection[63]. Patients treated with nerve allotransplantation alone require immunosuppression only for the time necessary for host axons to traverse the allograft and reach host motor and sensory targets. Similarly, experimental studies on acute rejection of nerve allografts have demonstrated only a very short window of time during which immunosuppression can rescue a nerve allograft undergoing acute rejection[64]. If there is an episode of acute rejection, it is only a matter of days before the nerve allograft can no longer be rescued.

Thus, episodes of acute rejection in hand transplant recipients are more than just temporary setbacks. Even though these acute episodes have been controlled relatively easily, the fact that they have been frequent and that some have occurred late predicts chronic rejection and ultimate failure of the allograft.

Balancing Risks and Benefits

Risks that are considered to be acceptable in association with solid organ transplantation are not necessarily acceptable in association with composite tissue allotransplantation. The benefits of each type of transplant surgery must be weighed against the concomitant risks. Table VII shows one scheme for classifying different types of transplant surgery. In the first group are lifesaving transplants—for example, heart, lung, or liver transplants. In the second group are transplants that, although not immediately lifesaving, alleviate chronic disease and offer the prospect of improved longevity and quality of life. Composite tissue allotransplantations are a third group. These transplants offer the possibility of functional improvement alone. The expected outcomes of surgery and subsequent immunosuppression differ for each of these groups, as shown in Table VIII. While all share the morbidity of a new disease (immunosuppression), the benefits of transplantation lie along a continuum. Cardiac, lung, and liver transplantations are lifesaving. A pancreas transplant to a diabetic patient or a kidney transplant to a patient with end-stage renal disease may prolong life. Recipients of kidney transplants from living relatives have been shown to survive longer than patients on dialysis[65]. In each of these examples, solid organ transplantation has transcended the biological limits of end-organ failure. Since composite tissue transplantation does not replace essential organs and remains experimental, a lower level of morbidity is acceptable. Also, unlike solid organ transplantation, which has an established track record of clinical success, composite tissue allotransplantation is still experimental.

Ethical Considerations in Determining Whether to Perform Experimental Surgery

What criteria can be used to determine whether to perform experimental surgery? Siegler[4] suggested that the six criteria originally proposed by Moore[66,67] be used for evaluating how innovative surgical techniques might be applied to hand transplantation. The criteria include (1) the scientific background of the innovation, (2) the skill and experience of the

TABLE VIII Risks and Benefits of Different Types of Transplant Surgery

Condition	Risks and Benefits of Transplantation
Life-threatening disease	Survival, but with chronic disease (immunosuppression), improved quality and quantity of life
Debilitating chronic disease	Reversal or alleviation of prior chronic disease, new chronic disease (immunosuppression)
Functional deficit without active disease	Long-term outcome unknown, new chronic disease (immunosuppression)

team (so-called field strength), (3) the ethical climate of the institution, (4) open display, (5) public evaluation, and (6) public and professional discussion.

These criteria address many aspects of experimental surgery—not only the scientific underpinnings and the ethical backdrop of the surgery, but also the practical issues of who will perform the surgery, how and where it will be done, and how it will be evaluated. Siegler[4] concluded that the teams working on clinical hand transplantation had satisfactorily met these criteria, and he recommended proceeding with cadaveric hand transplant surgery in humans. However, it bears mention that these criteria were designed as ethical standards for the use of innovative surgery for life-threatening, otherwise untreatable conditions, specifically the use of innovative liver transplantation at the University of Chicago[68].

While criteria 2 through 6 can reasonably be extrapolated from liver transplantation to hand transplantation and other types of composite tissue transplantation, the same does not hold for criterion 1. The central concept in criterion 1 is the equipoise consideration. *Equipoise* refers to a clinical setting in which the prospects for benefiting the patient are favorably balanced against the risk of causing unintended harm. The threshold for undertaking innovative surgery is necessarily much higher for hand transplantation, the goal of which is restoration of function, than for liver transplantation for the treatment of a life-threatening condition. For hand transplantation to meet equipoise conditions, the benefits in terms of functional improvement and enhanced quality of life from the patient's perspective must equal or exceed the risks of the surgery and subsequent immunosuppression.

Siegler's[4] assessment of the equipoise criterion here warrants scrutiny:

"Is it ethically acceptable to allow a patient to balance an improvement in quality of life (such as may be obtained from hand transplant) against the potential risk of morbidity and mortality. . . ? Specifically, is improving the patient's quality of life (as determined by the patient) sufficient grounds to allow patients to risk morbidity and mortality? The answer is clearly 'yes' because such trade-offs are inevitable and are not unique to hand transplantation."

The answer is "*clearly* 'yes'," only when benefits *clearly* exceed risks. Physicians and surgeons regularly accept risk as the necessary price of conferring future benefits. Although an experimental treatment may provide benefit from the patient's perspective, it is the surgeon's responsibility to make sure that such benefit does not exact an unacceptable cost. Before undertaking experimental surgery, it behooves us to reflect on the tenet "primum non nocere" ("first, do no harm").

The Principle-Based Approach to Human Experimentation

Several incentives may drive surgeons to move forward with new techniques faster than is appropriate. Among these are desperate patients, the patient's or surgeon's desire for notoriety, and institutional interest in market share. As Selzer wrote: "In the act of surgery, the scalpel must be restrained rather than given its head. Holding back is the primary mode of surgery."[69] The ethical precepts derived from the Declaration of Helsinki and from the Nuremberg trials can help to steady our hand[70,71]. The Nuremberg Code advises in part:

> (2) The experiment should be such as to yield fruitful results for the good of society, unprocurable by other methods or means of study. . . .
>
> (3) The experiment should be so designed and based on the results of animal experimentation. . . .
>
> (10) During the course of the experiment the scientist in charge must be prepared to terminate the experiment at any stage, if he has probable cause to believe, in the exercise of the good faith, superior skill and careful judgment required of him that a continuation of the experiment is likely to result in injury, disability, or death to the experimental subject.

The following excerpts from the Helsinki Declaration are also particularly relevant:

> (1) Biomedical research involving human subjects . . . should be based on adequately performed laboratory and animal experimentation. . . .
>
> (3) . . . The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.
>
> (5) . . . Concern for the interests of the subject must always prevail over the interests of science and society.

Based on the principles from both Nuremberg and Helsinki, several aspects of hand transplantation raise concerns. Both sets of principles place special emphasis on conducting thorough animal research prior to human experimentation. While some animal models of composite tissue allotransplantation do exist, they have shown varying levels of success[13-15]. Specifically, the pooled data from three primate studies indicated that rejection had occurred in eighteen of twenty-four animals[72,73]. Sepsis or malignant disease developed in fifteen of those animals, and only two of the twenty-four animals survived beyond 200 days. As Lee and Mathes pointed out, no animal studies have successfully demonstrated return of function following limb allotransplantation[7].

Both the Nuremburg Code and the Helsinki Declaration instruct us to safeguard the welfare of the individual patient, a responsibility that begins before surgery takes place. Principle 3 of the Helsinki Declaration suggests that the surgeon is obliged to balance thoughtful guidance with careful attention to the patient's needs. While it is inappropriate to impose personal values on patients, it is equally unacceptable to offer patients an innovative treatment and then sit back in silence as he or she agonizes, alone, over a final decision. Principle 10 of the Nuremberg Code emphasizes the need to terminate an experiment once there is indication that it may be injurious to the patient.

As Principle 5 of the Helsinki Declaration emphasizes, human experimentation that purports to serve the "greater

good" at the expense of the individual is not justifiable. The long-term success of clinical hand transplantation remains uncertain. While hand replantation sometimes results in durable recovery of function[17], hand transplantation has not yet been shown to provide such favorable outcomes. This must be taken into account when determining whether surgery is in the patient's best interest.

Assessment with use of quality-adjusted life-years could theoretically assist in decision-making regarding hand transplantation. This method provides a quantitative interpretation of the subjective risks and benefits of a treatment. Unfortunately, in the case of hand transplantation, the principal benefits are not certain since, as Lee and Mathes[7] pointed out, long-term survival of limb allografts has not been achieved. Whether functional recovery can be sustained long-term after human hand transplantation is unknown. Furthermore, the psychological repercussions of a favorable or unfavorable outcome are difficult to quantify. The world's first "successful" hand transplant was amputated on February 3, 2001. Among the patient's complaints were the adverse effects of the treatment regimen, poor hand function, deterioration of the hand's physical appearance, and a sense of both physical and psychic detachment from the transplanted hand. In the wake of this treatment failure, the impact of rejection on quality of life must not be taken lightly[74].

Given the morbidity associated with chronic immunosuppression and the lack of evidence supporting durable recovery of function, it would seem that additional research and reflection are warranted before proceeding with additional hand transplants. It seems doubtful that hand transplantation in its current state serves the best interests of the individual transplant recipient. How many members of the hand or transplant surgery community would be willing to undergo a hand transplant were they to lose one of their hands?

Future Directions

Composite tissue allotransplantation has become a source of not only tremendous enthusiasm, but also considerable controversy. Nonetheless, composite tissue allotransplantation remains one of the great frontiers in hand and reconstructive surgery. Progress in developing models of immune tolerance suggests that a more promising paradigm for composite tissue allotransplantation may be forthcoming. Immune tolerance is a state of donor-specific immune unresponsiveness leading to the indefinite engraftment of transplanted tissue without the need for ongoing therapy. Strategies of tolerance induction include irradiation, donor-bone-marrow transfusion, intrathymic injection of donor cells, and antibody-based therapies. Among the promising antibody-based therapies are T-cell depletion, blockade of antigen recognition, blockade of adhesion molecules, and costimulation blockade. Currently, several tolerance-inducing strategies, including donor-bone-marrow transfusion with immunosuppression, anti-ICAM (intercellular adhesion molecule)-1 antibody, anti-LFA (lymphocyte function-associated)-1 monoclonal antibody, anti-CD25 monoclonal antibody, and anti-CD40 ligand monoclonal antibody, are being studied in clinical trials[75].

Blockade of the CD40 costimulatory pathway appears to be one of the most promising approaches and has produced long-term donor-specific allograft survival in many experimental models with minimal toxicity[76-83]. Its role in the future of transplantation seems to be as part of a combined regimen that includes other modalities such as T-cell depletion and bone-marrow transfusion[84-86]. However, the enthusiasm resulting from the efficacy of CD40L blockade in models of limb[87] and nerve allotransplantation[64] must be tempered with the possibility that the potent neuroregenerative effects of tacrolimus cannot be used in combination with costimulation blockade. When used simultaneously, calcineurin inhibitors such as tacrolimus and cyclosporine A have been shown to abrogate the effects of costimulation blockade in numerous models of skin and solid organ transplantation[88-92].

The universal acceptance of composite tissue allotransplantation as a safe and viable therapeutic option rests with the reduction or elimination of the adverse effects of immune modulation. The successful induction of donor-specific tolerance would circumvent the toxicity associated with nonspecific immunosuppression and may eliminate the potential for chronic rejection. The result would be the safe transfer of composite tissue allografts leading to improved functional outcomes and reduced morbidity.

Michael J. Brenner, MD
Thomas H. Tung, MD
John N. Jensen, MD
Susan E. Mackinnon, MD
Division of Plastic and Reconstructive Surgery, Washington University School of Medicine, One Barnes-Jewish Hospital Plaza, Suite 17424, East Pavilion, St. Louis, MO 63110. E-mail address for T.H. Tung: tungt@msnotes.wustl.edu. E-mail address for S.E. Mackinnon: mackinnons@msnotes.wustl.edu

The authors did not receive grants or outside funding in support of their research or preparation of this manuscript. They did not receive payments or other benefits or a commitment or agreement to provide such benefits from a commercial entity. No commercial entity paid or directed, or agreed to pay or direct, any benefits to any research fund, foundation, educational institution, or other charitable or nonprofit organization with which the authors are affiliated or associated.

Read in part at the Annual Meeting of the American Society of Plastic Surgeons, Orlando, Florida, November 2001.

References

1. **Cecka JM.** The UNOS Scientific Renal Transplant Registry—ten years of kidney transplants. *Clin Transplants.* 1997:1-14.
2. **Llull R, Beko KR II, Black KS, Hewitt CW.** Composite tissue allotransplantation: perspectives concerning eventual clinical exploitation. *Transplant Rev.* 1992;6:175-88.
3. **Llull R.** An open proposal for clinical composite tissue allotransplantation. *Transplant Proc.* 1998;30:2692-703.

4. **Siegler M.** Ethical issues in innovative surgery: should we attempt a cadaveric hand transplantation in a human subject? *Transplant Proc.* 1998;30:2779-82.

5. **Hewitt CW.** Update and outline of the experimental problems facing clinical composite tissue transplantation. *Transplant Proc.* 1998;30:2704-7.

6. **Francois CG, Breidenbach WC, Maldonado C, Kakoulidis TP, Hodges A, Dubernard JM, Owen E, Pei G, Ren X, Barker JH.** Hand transplantation: comparisons and observations of the first four clinical cases. *Microsurgery.* 2000;20:360-71.

7. **Lee WP, Mathes DW.** Hand transplantation: pertinent data and future outlook. *J Hand Surg [Am].* 1999;24:906-13.

8. **Cooney WP, Hentz VR.** Successful hand transplantation—one-year follow-up [letter]. *N Engl J Med.* 2001;344:65.

9. **Foucher G.** Prospects for hand transplantation [letter]. *Lancet.* 1999;353: 1286-7.

10. **Dubernard JM, Owen ER, Lanzetta M, Hakim N.** What is happening with hand transplants [letter]. *Lancet.* 2001;357:1711-2.

11. **Manske PR.** Hand transplantation [editorial]. *J Hand Surg [Am].* 2001;26: 193-5.

12. **Hettiaratchy S, Butler PE, Lee WP.** Lessons from hand transplantations. *Lancet.* 2001;357:494-5.

13. **Jensen JN, Mackinnon SE.** Composite tissue allotransplantation: a comprehensive review of the literature–part I. *J Reconstr Microsurg.* 2000;16:57-68.

14. **Jensen JN, Mackinnon SE.** Composite tissue allotransplantation: a comprehensive review of the literature–Part II. *J Reconstr Microsurg.* 2000;16:141-57.

15. **Jensen JN, Mackinnon SE.** Composite tissue allotransplantation: a comprehensive review of the literature–part III. *J Reconstr Microsurg.* 2000;16:235-51.

16. **Zhang J, Chen ZW.** Retrospective of the replantation of severed limbs in the People's Republic of China: current status and prospects. *Microsurgery.* 2002;22:39-43.

17. **Buncke HJ Jr.** Microvascular hand surgery - transplants and replants - over the past 25 years. *J Hand Surg [Am].* 2000;25:415-28.

18. **Strome M, Stein J, Esclamado R, Hicks D, Lorenz RR, Braun W, Yetman R, Eliachar I, Mayes J.** Laryngeal transplantation and 40-month follow-up. *N Engl J Med.* 2001;344:1676-9.

19. **Strome M.** Human laryngeal transplantation: considerations and implications. *Microsurgery.* 2000;20:372-4.

20. **Haughey BH, Beggs JC, Bong J, Genden EM, Buckner A.** Microneurovascular allotransplantation of the canine tongue. *Laryngoscope.* 1999;109:1461-70.

21. **Altman LK.** A short, speckled history of a transplanted hand. *New York Times*. 2001 Feb 27;Science Section:1.

22. **Goldstein LS, Haug MT 3rd, Perl J 2nd, Perl MK, Maurer JR, Arroglia AC, Mehta AC, Kirby T, Higgins B, Stillwell PC.** Central nervous system complications after lung transplantation. *J Heart Lung Transplant.* 1998;17:185-91.

23. **Bronster DJ, Emre S, Mor E, Sheiner P, Miller CM, Schwartz ME.** Neurologic complications of orthotopic liver transplantation. *Mt Sinai J Med.* 1994;61:63-9.

24. **Hoekstra HJ, Hawkins K, de Boer WJ, Rottier K, van der Bij W.** Gastrointestinal complications in lung transplant survivors that require surgical intervention. *Br J Surg.* 2001;88:433-8.

25. **Maurer JR.** The spectrum of colonic complications in a lung transplant population. *Ann Transplant.* 2000;5:54-7.

26. **Dummer JS, Hardy A, Poorsattar A, Ho M.** Early infections in kidney, heart, and liver transplant recipients on cyclosporine. *Transplantation.* 1983;36: 259-67.

27. **Snydman DR.** Epidemiology of infections after solid-organ transplantation. *Clin Infect Dis.* 2001;33 Suppl 1:S5-8.

28. **Patel R, Paya CV.** Infections in solid-organ transplant recipients. *Clin Microbiol Rev.* 1997;10:86-124.

29. **Porter GA, Bennett WM, Sheps SG.** Cyclosporine-associated hypertension. National High Blood Pressure Education Program. *Arch Intern Med.* 1990; 150:280-3.

30. **Yamani MH, Starling RC.** Long-term medical complications of heart transplantation: information for the primary care physician. *Cleve Clin J Med.* 2000;67:673-80.

31. **Hauters P, de Hemptinne B, Carlier M, Malaise J, Ottobrelli A, Delaby J, Balducci G, Kestens PJ, Otte B.** Long-term analysis of glomerular filtration rate and hypertension in adult liver transplant recipients treated with cyclosporine A. *Transplant Proc.* 1991;23:1458-9.

32. **Canzanello VJ, Textor SC, Taler SJ, Schwartz LL, Porayko MK, Wiesner RH, Krom RA.** Late hypertension after liver transplantation: a comparison of cyclosporine and tacrolimus (FK 506). *Liver Transpl Surg.* 1998;4:328-34.

33. **Jain A, Reyes R, Kashyap R, Rohal S, Cacclarelli T, McMichael J, Rakela J, Starzl TE, Fung JJ.** Liver transplantation under tacrolimus in infants, children, adults, and seniors: long-term results, survival, and adverse events in 1000 consecutive patients. *Transplant Proc.* 1998;30:1403-4.

34. **Cronin DC 2nd, Faust TW, Brady L, Conjeevaram H, Jain S, Gupta P, Millis JM.** Modern immunosuppression. *Clin Liver Dis.* 2000;4:619-55, ix.

35. **Zietse R, Balk AH, Dorpel MA, Meeter K, Bos E, Weimar W.** Time course of the decline in renal function in cyclosporine-treated heart transplant recipients. *Am J Nephrol.* 1994;14:1-5.

36. **Woolfson RG, Neild GH.** Cyclosporin nephrotoxicity following cardiac transplantation. *Nephrol Dial Transplant.* 1997;12:2054-6.

37. **Myers BD, Newton L.** Cyclosporine-induced chronic nephropathy: an obliterative microvascular renal injury. *J Am Soc Nephrol.* 1991;2(2 Suppl 1):S45-52.

38. **Asante-Korang A, Boyle GJ, Webber SA, Miller SA, Fricker FJ.** Experience of FK506 immune suppression in pediatric heart transplantation: a study of long-term adverse effects. *J Heart Lung Transplant.* 1996;15:415-22.

39. **Ramsey-Goldman R, Dunn JE, Dunlop DD, Stuart FP, Abecassis MM, Kaufman DB, Langman CB, Salinger MH, Sprague SM.** Increased risk of fracture in patients receiving solid organ transplants. *J Bone Miner Res.* 1999;14:456-63.

40. **Shane E, Epstein S.** Transplantation osteoporosis. *Transpl Rev.* 2001; 15:11-32.

41. **Guckelberger O, Langrehr JM, Bechstein WO, Neuhaus R, Luesebrink R, Lemmens HP, Kratschmer B, Jonas S, Knoop M, Neuhaus P.** Does the choice of primary immunosuppression influence the prevalence of cardiovascular risk factors after liver transplantation? *Transplant Proc.* 1996;28:3173-4.

42. **Hosenpud JD, Bennett LE, Keck BM, Boucek MM, Novick RJ.** The Registry of the International Society for Heart and Lung Transplantation: eighteenth Official Report-2001. *J Heart Lung Transplant.* 2001;20:805-15.

43. **Jindal RH.** Posttransplant diabetes mellitus—a review. *Transplantation.* 1994;58:1289-98.

44. **Weir MR, Fink JC.** Risk for postransplant diabetes mellitus with current immunosuppressive medications. *Am J Kidney Dis.* 1999;34:1-13.

45. **Danovitch GM.** Immunosuppressant-induced metabolic toxicities. *Transplant Rev.* 2000;14:65-81.

46. **Penn I.** Posttransplant malignancies. *Transplant Proc.* 1999;31:1260-2.

47. **Otley CC, Pittelkow MR.** Skin cancer in liver transplant recipients. *Liver Transpl.* 2000;6:253-62.

48. **Bouwes Bavinck JN, Hardie DR, Green A, Cutmore S, MacNaught A, O'Sullivan B, Siskind V, Van Der Woude FJ, Hardie IR.** The risk of skin cancer in renal transplant recipients in Queensland, Australia. A follow-up study. *Transplantation.* 1996;61:715-21.

49. **Hartevelt MM, Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP.** Incidence of skin cancer after renal transplantation in The Netherlands. *Transplantation.* 1990;49:506-9.

50. **Penn I.** Post-transplant malignancy: the role of immunosuppression. *Drug Saf.* 2000;23:101-13.

51. **Sheil AG, Disney AP, Mathew TH, Amiss N.** De novo malignancy emerges as a major cause of morbidity and late failure in renal transplantation. *Transplant Proc.* 1993;25:1383-4.

52. **Kwok BW, Hunt SA.** Neoplasia after heart transplantation. *Cardiol Rev.* 2000;8:256-9.

53. **Ong CS, Keogh AM, Kossard S, Macdonald PS, Spratt PM.** Skin cancer in Australian heart transplant recipients. *J Am Acad Dermatol.* 1999;40:27-34.

54. **Preiksaitis JK, Keay S.** Diagnosis and management of posttransplant lymphoproliferative disorder in solid-organ transplant recipients. *Clin Infect Dis.* 2001;33 Suppl 1:S38-46.

55. **Cohen AH, Sweet SC, Mendeloff E, Mallory GB Jr, Huddleston CB, Kraus M, Kelly M, Hayashi R, DeBaun MR.** High incidence of posttransplant lymphoproliferative disease in pediatric patients with cystic fibrosis. *Am J Respir Crit Care Med.* 2000;161:1252-5.

56. **Finn L, Reyes J, Bueno J, Yunis E.** Epstein-Barr virus infections in children after transplantation of the small intestine. *Am J Surg Pathol.* 1998;22:299-309.

57. **Womer KL, Lee RS, Madsen JG, Sayegh MH.** Tolerance and chronic rejection. *Philos Trans R Soc Lond B Biol Sci.* 2001;356:727-38.

58. **Gao SZ, Schroeder JS, Alderman EL, Hunt SA, Valantine HA, Wiederhold V,**

Stinson EB. Prevalence of accelerated coronary artery disease in heart transplant survivors. Comparison of cyclosporine and azathioprine regimens. *Circulation.* 1989;80:III100-5.

59. **Orloff MS, DeMara EM, Coppage ML, Leong N, Fallon MA, Sickel J, Zuo XJ, Prehn J, Jordan SC.** Prevention of chronic rejection and graft arteriosclerosis by tolerance induction. *Transplantation.* 1995;59:282-8.
60. **Cecka JM, Terasaki PI.** The UNOS Scientific Renal Transplant Registry. In: Terasaki PI, Cecka JM, editors. *Clinical transplants 1994*. Los Angeles: UCLA Tissue Typing Laboratory; 1994. p 1-18.
61. **Tejani A, Sullivan EK.** The impact of acute rejection on chronic rejection: a report of the North American Pediatric Renal Transplant Cooperative Study. *Pediatr Transplant.* 2000;4:107-11.
62. **Zhong-Wei C, Meyer VE, Kleinert HE, Beasley RW.** Present indications and contraindications for replantation as reflected by long-term functional results. *Orthop Clin North Am.* 1981;12:849-70.
63. **Jensen JN, Lowe JB, Brenner MJ, Sen S, Grand A, Myckatyn T, Hunter DA, Mackinnon SE.** Anti-CD40 ligand antibody permits regeneration through peripheral nerve allografts in a non-human primate model. Unpublished data.
64. **Feng FY, Ogden MA, Myckatyn TM, Grand AG, Jensen JN, Hunter DA, Mackinnon SE.** FK506 rescues peripheral nerve allografts in acute rejection. *J Neurotrauma.* 2001;18:217-29.
65. **Vollmer WM, Wahl PW, Blagg CR.** Survival with dialysis and transplantation in patients with end-stage renal disease. *N Engl J Med.* 1983;308:1553-8.
66. **Moore FD.** Three ethical revolutions: ancient assumptions remodeled under pressure of transplantation. *Transplant Proc.* 1988;20(1 Suppl 1):1061-7.
67. **Moore FD.** The desperate case: CARE (costs, applicability, research, ethics) [editorial]. *JAMA.* 1989;261:1483-4.
68. **Singer PA, Siegler M, Whitington PF, Lantos JD, Emond JC, Thistlethwaite JR, Broelsch CE.** Ethics of liver transplantation with living donors. *N Engl J Med.* 1989;321:620-2.
69. **Selzer R.** *Mortal lessons: notes on the art of surgery*. New York: Harcourt Brace; 1996. p 8.
70. *Trials of war criminals before the Nuremberg Military Tribunals under Control Council Law.* No 10, vol 2. p 181-2. ohsr.od.nih.gov/nuremberg.php3
71. *World Medical Association Declaration of Helsinki.* ohsr.od.nih.gov/helsinki.php3
72. **Daniel RK, Egerszegi EP, Samulack DD, Skanes SE, Dykes RW, Rennie WR.** Tissue transplants in primates for upper extremity reconstruction: a preliminary report. *J Hand Surg [Am].* 1986;11:1-8.
73. **Stark GB, Swartz WM, Narayanan K, Moller AR.** Hand transplantation in baboons. *Transplant Proc.* 1987;19:3968-71.
74. Hand transplant recipient throws in the towel. *Hastings Center Report.* 2002;31:6-7.
75. **Aradhye S, Turka LA.** Will tolerance become a clinical reality? *Am J Med Sci.* 1997;313:310-4.
76. **Larsen CP, Alexander DZ, Hollenbaugh D, Elwood ET, Ritchie SC, Aruffo A, Hendrix R, Pearson TC.** CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation.* 1996;61:4-9.
77. **Kenyon NS, Fernandez LA, Lehmann R, Masetti M, Ranuncoli A, Chatzipetrou M, Iaria G, Han D, Wagner JL, Ruiz P, Inverardi L, Alejandro R, Mintz DH, Kirk AD, Harlan DM, Burkly LC, Ricordi C.** Long-term survival and function of intrahepatic islet allografts in baboons treated with humanized anti-CD154. *Diabetes.* 1999;48:1473-81.
78. **Kenyon NS, Chatzipetrou M, Masetti M, Ranuncoli A, Oliveira M, Wagner JL, Kirk AD, Harlan DM, Burkly LC, Ricordi C.** Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. *Proc Natl Acad Sci U S A.* 1999;96:8132-7.
79. **Knechtle SJ, Kirk AD, Fechner JH Jr, Hong X, Dong Y, Hamawy MM, Harlan DM.** Inducing unresponsiveness by the use of anti-CD3 immunotoxin, CTLA4-Ig, and anti-CD40 ligand. *Transplant Proc.* 1999:31(3B Suppl):27S-8S.
80. **Kirk AD.** Transplantation tolerance: a look at the nonhuman primate literature in the light of modern tolerance theories. *Crit Rev Immunol.* 1999;19:349-88.
81. **Larsen CP, Pearson TC.** The CD40 pathway in allograft rejection, acceptance, and tolerance. *Curr Opin Immunol.* 1997;9:641-7.
82. **Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH Jr, Knechtle SJ.** CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci U S A.* 1997;94:8789-94.
83. **Durham MM, Bingaman AW, Adams AB, Ha J, Waitze SY, Pearson TC, Larsen CP.** Cutting edge: administration of anti-CD40 ligand and donor bone marrow leads to hemopoietic chimerism and donor-specific tolerance without cytoreductive conditioning. *J Immunol.* 2000;165:1-4.
84. **Wekerle T, Kurtz J, Ito H, Ronquillo JV, Dong V, Zhao G, Shaffer J, Sayegh MH, Sykes M.** Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. *Nat Med.* 2000;6:464-9.
85. **Russell PS, Chase CM, Sykes M, Ito H, Shaffer J, Colvin RB.** Tolerance, mixed chimerism, and chronic transplant arteriopathy. *J Immunol.* 2001;167:5731-40.
86. **Ito H, Kurtz J, Shaffer J, Sykes M.** CD4 T cell-mediated alloresistance to fully MHC-mismatched allogeneic bone marrow engraftment is dependent on CD40-CD40 ligand interactions, and lasting T cell tolerance is induced by bone marrow transplantation with initial blockade of this pathway. *J Immunol.* 2001;166:2970-81.
87. **Tung TH, Mackinnon SE, Mohankumar T.** Long-term limb allograft survival using anti-CD40 L antibody in a murine model. Unpublished data.
88. **Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB.** Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nat Med.* 1999;5:1298-302.
89. **Li Y, Zheng XX, Li XC, Zand MS, Strom TB.** Combined costimulation blockade plus rapamycin but not cyclosporine produces permanent engraftment. *Transplantation.* 1998;66:1387-8.
90. **Smiley ST, Csizmadia V, Gao W, Turka LA, Hancock WW.** Differential effects of cyclosporine A, methylprednisolone, mycophenolate, and rapamycin on CD154 induction and requirement for NFkappaB: implications for tolerance induction. *Transplantation.* 2000;70:415-9.
91. **Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C, Cho HR, Aruffo A, Hollenbaugh D, Linsey PS, Winn KJ, Pearson TC.** Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature.* 1996;381:434-8.
92. **Kirk AD, Burkly LC, Batty DS, Baumgartner RE, Berning JD, Fechner JH Jr, Germond RL, Kampen RL, Patterson NB, Swanson SJ, Tadaki SK, TenHoor CN, White L, Knechtle SJ, Harlan DM.** Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nat Med.* 1999;5:686-93.
93. **Restrepo A, Albrecht F, Raez LE, Fernandez HF, Nassiri M, Byrne G Jr, Cassileth PA.** Post-liver transplantation lymphoproliferative disorders with and without infusions of donor bone marrow cells. *Crit Rev Oncog.* 1999;10:239-45.
94. **Fahlenkamp D, Reinke P, Kirchner S, Schnorr D, Lindeke A, Loening SA.** Malignant tumours after renal transplantation. *Scand J Urol Nephrol.* 1996;30:357-62.
95. **Ying AJ, Myerowitz PD, Marsh WL Jr.** Posttransplantation lymphoproliferative disorder in cardiac transplant allografts. *Ann Thorac Surg.* 1997;64:1822-4.
96. **Gjersvik P, Hansen S, Moller B, Leivestad T, Geiran O, Simonsen S, Pfeffer P, Fauchald P.** Are heart transplant recipients more likely to develop skin cancer than kidney transplant recipients? *Transplant Int.* 2000;13 Suppl 1:S380-1.
97. **Jensen P, Hansen S, Moller B, Leivestad T, Pfeffer P, Geiran O, Fauchald P, Simonsen S.** Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. *J Am Acad Dermatol.* 1999;40:177-86.
98. **Haagsma EB, Hagens VE, Schaapveld M, van den Berg AP, de Vries EG, Klompmaker IJ, Sloof MJ, Jansen PL.** Increased cancer risk after liver transplantion: a population-based study. *J Hepatol.* 2001;34:89-91.