

Tolerance and the “Holy Grail” of Transplantation

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Advances in transplantation biology have greatly improved patient outcomes following transplant surgery. However, generalized immunosuppression remains the Achilles heel of modern transplantation surgery with its associated infectious and neoplastic morbidities. Tolerance remains the ultimate goal for the entire field. Although recent advances in transplant immunology suggest that tolerance may be achievable in the near future, the complex and redundant nature of the human immune system may not allow us to circumvent such a basic function as the recognition of nonself. In this paper, advances in transplant immunology are reviewed and their potential relevance to achieving the “Holy Grail” of transplantation are discussed. © 2003 Elsevier Inc. All rights reserved.

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INTRODUCTION

During the last 30 years, solid organ transplantation has become an accepted form of therapy for end-stage disease of many organs, including kidney, liver, heart, and lung. With the advent of new immunosuppressive therapies combining decreased toxicity and improved efficacy, patient survival and short-term (1- and 5-year) graft survival have markedly improved. However, the long-term survival of grafts that are functional at 1 year after transplant remains largely unchanged. As an example, the half-life of cadaveric renal transplants remains 8 to 10 years [1]. In addition, there remains significant patient morbidity and mortality from the increased risk of infection and neoplasia associated with current immunosuppressive regimens. As a result, there remains a need for alternatives to the

current globally immunosuppressive agents. Particularly attractive is the notion of inducing the immune system to establish antigen-specific tolerance to transplant or allograft antigens.

Transplantation tolerance has long been the “Holy Grail” for transplant immunologists, physicians and surgeons. There are multiple definitions. For the purposes of this clinical management module, tolerance is defined as immune unresponsiveness in the absence of ongoing therapy to graft alloantigens, but not to other (third-party) antigens. The functional characteristics of tolerance are: 1) lack of demonstrable immune reactivity to graft alloantigens; 2) presence of immune reactivity to other alloantigens; and 3) absence of generalized immunosuppression for graft maintenance [2]. In the clinical setting, a tolerant individual retains a functional graft, retains immune reactivity to all other foreign antigens, and avoids the risks of generalized immunosuppression.

Until very recently, a clinically applicable strategy for transplantation tolerance was elusive. However, new understanding of the mechanisms of antigen recognition and development of specific reagents has attracted the interest of those in the transplant community, industry and the National Institutes of Health (NIH). In February 1998, an expert panel convened by the NIH concluded that a solid experimental foundation already existed and many unique reagents were available to support translational research and pursue clinical applications [3]. It appears that transplantation tolerance is on the verge of applicability toward clinical organ transplantation. Starzl describes the evolution of modern transplant immunology and the emergence of conditions that result in immunologic tolerance in his article on transplantation tolerance from a historical perspective [4]. He describes mechanisms of nonreactivity that result in allograft tolerance as emanating from initial, post-transplant, interactions between donor and recipient immune cells, which

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result in clonal exhaustion and eventual peripheral deletion. One explanation for the observed deletion associated apoptosis may result from a Fas–Fas ligand interaction, which results in cell death.

He also refers to immune ignorance as a state in which antigen is ignored by a host immune system because the antigen fails to reach the organized lymphoid collections where recognition of non-self is initiated. Because antigens must migrate to draining lymph nodes to generate antigen recognition, allografts lose immunogenicity when their passenger leukocytes are depleted and no longer capable of migrating to host lymphoid organs [4]. It is known that foreign antigens must be processed and carried to lymph nodes for the initiation of an immune response. Chemokine receptors and adhesion molecules are critical to the migration, of the antigen bearing cells, from the circulation into lymph nodes. Therefore, blockade of these receptor molecules may result in a state of “immune ignorance,” leading to tolerance. Prevention of either antigen recognition or the array of co-stimulatory interactions that follows antigen presentation may result in anergy. In this module, we will review the basics of transplantation immunology, the history of tolerance research, recent developments, and the implications of these studies for the future of transplantation tolerance in the clinical setting.

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REVIEW OF TRANSPLANT IMMUNOLOGY

Classically, transplantation rejection is classified into three categories: hyperacute, acute, and chronic [2]. Hyperacute rejection is mediated by the presence of preexisting antibodies to blood types O, A, or B; human leukocyte antigens; or other polymorphic antigens. In this setting, antibodies immediately bind and activate complement. Platelets and fibrin are deposited, granulocytes and monocytes infiltrate the graft, and fibrinoid necrosis of the vessel wall results. Typically, ischemic necrosis of the allograft occurs within 18–24 h. These preformed antibodies occur as the result of previous sensitization through transfusions, pregnancy, or bacterial infections that induce crossreacting antibodies. This form of rejection has largely been eliminated as the result of routine preoperative cross-matches.

Acute rejection is based upon recognition of foreign tissue as nonself. The signaling pathway for self vs nonself resides in a series of cell-surface glycoproteins that comprise the major histocompatibility complex (MHC). Alleles of MHC proteins are highly polymorphic. As a result, alloantigenicity and recognition of

nonself resides in MHC disparity, which incites activation of the host immune response. After activation of recipient immune cells, macrophages secrete cytokines, such as transforming growth factor- β , platelet-derived growth factor, interleukin (IL)-1 and tumor necrosis factor (TNF) [5]. These in turn induce expression of chemokines, such as IL-8, monocyte chemoattractant proteins 1–3, and RANTES [5]. IL-8 serves as a potent chemoattractant for granulocytes, whereas monocyte chemoattractant protein attracts macrophages and RANTES attracts monocytes and T cells [5]. Inducible cell surface receptors are expressed on graft vessels, leading to margination and rolling of immune cells along the vascular endothelium. Haptotaxis and chemotaxis result. The end result of these processes is graft destruction in the setting of a cellular infiltrate of monocytes, lymphocytes, and the variable presence of eosinophils, granulocytes, macrophages, and natural killer cells. Immunosuppression and immune tolerance strategies currently focus primarily upon abolition of acute rejection after transplantation.

The last form of graft rejection occurs months to years after transplantation. Chronic rejection occurs even in the setting of continued immunosuppression [6]. It is characterized by fibrosis with distortion of the normal cellular architecture of the organ, in a fashion similar to that which accompanies wound healing. Although the etiology of chronic rejection remains unclear, multiple mechanisms have been suggested: healing in response to recurrent episodes of acute rejection, delayed-type hypersensitivity, ultimately resulting in macrophage activation and release of tissue growth factors, antibody-mediated humoral rejection, and endothelial injury, leading to ischemia. Chronic rejection is a major cause of late allograft loss, and current immunosuppressive therapies do not address nor reverse chronic rejection [7].

Host Immune Response to Allografts

Transplanted organs express donor MHC molecules, which results in two pathways for antigen recognition (Fig. 1). In the direct pathway, recipient T cells recognize allogeneic MHC molecules expressed on donor cells. Donor origin antigen presenting cells (APCs) constitutively express MHC class I molecules that continuously express self peptides. These intact allogeneic MHC molecules are recognized because they resemble self MHC plus foreign peptide in tertiary structure, so-called molecular mimicry. In addition, coactivation molecules, such as CD40 and B7, are constitutively expressed. Alloantigenic peptides presented by donor class I MHC molecules appear to be directly engaged by CD8+ T cells. These recipient T cells also receive coactivation signals, become activated, and proceed to destroy the target cells. In the indirect pathway, peptides derived from degraded donor MHC molecules are

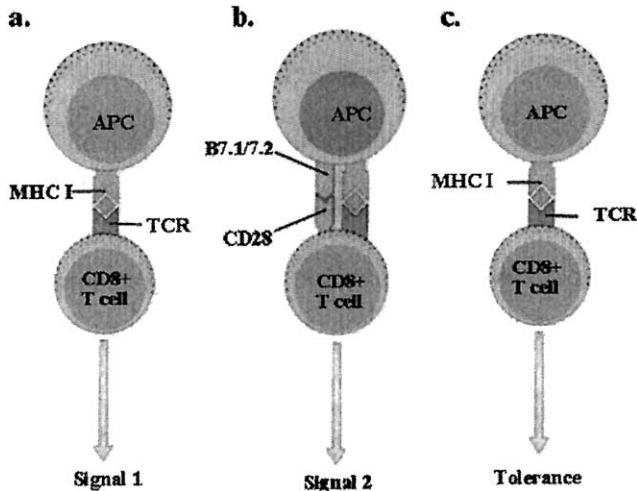


FIG. 1. (a) The first step in antigen recognition is referred to as signal one and involves recognition, by the T cell receptor, of a peptide lodged in the peptide binding groove of the major histocompatibility complex. (b) The second step involves binding of costimulatory molecules, B7.1 and B7.2, by CD 28, which results in activation and clonal expansion of T cells. This is referred to as signal two. (c) Presentation of an antigen in the presence of MHC to the TCR in the absence of costimulation may lead to tolerance.

presented by self MHC on recipient APC. This form of antigen presentation by MHC class II molecules is directed toward host CD4⁺ T cells. The activation of T cells by the indirect pathway is less efficient than that of T cells primed by the direct pathway. The T cell repertoire appears to be restricted after indirect antigen presentation. It has been postulated that the direct pathway mediates the vigorous immune response seen in acute rejection, whereas the indirect pathway may have the dominant role in chronic rejection [2, 6].

Ligation of the T cell receptor (TCR) activates multiple intracellular signaling events. The earliest events include removal of inhibitory phosphate groups by CD45 tyrosine phosphatase, resultant activation of the Lck and Fyn tyrosine kinases associated with the CD4/CD8 coreceptor and TCR-CD3 complex, respectively, and phosphorylation of the TCR zeta-chain. This phosphorylation recruits and activates the zeta-associated protein-70 (ZAP-70), which results in three important signaling events. Two of these involve activation of phospholipase C- γ , which cleaves phosphatidylinositol into diacylglycerol and inositol trisphosphate. Diacylglycerol activates protein kinase C, which activates the transcription factor NF- κ B. Inositol trisphosphate increases intracellular calcium concentrations, which activates the phosphatase calcineurin, inducing the transcription factor NF-AT. The third important signaling event is the activation of Ras. Kinases downstream of Ras, including extracellular signal-regulated kinase, ultimately induce and activate Fos, a component of the AP-1 transcription factor.

Activation of the T cell then follows if simultaneous costimulation has occurred [5, 8].

Full activation of T cells requires two distinct signals [2, 6, 8]. The first is delivered through the TCR, as described above, and conveys the specificity of the immune response as it is antigen-based. The second or costimulatory signal is not antigen specific and may arise from a number of T cell molecules (Fig. 2). One of the more extensively characterized, the CD28 molecule, has two known ligands, B7-1 (CD80) and B7-2 (CD86); these are expressed primarily upon activated APC. T cells also express CTLA-4, which also binds to B7-1/2 and transmits an inhibitory signal to terminate the immune response. Finally, the CD40-CD40 ligand (CD154) pathway is also key for T cell activation. CD40 is expressed on many cell types, including APC and endothelial cells. Its ligand, CD154, is expressed upon activated CD4⁺ T cells. Activation of APC through CD40 induces B7 expression, and elaboration of adhesion molecules and cytokines that participate in T cell activation. In the absence of costimulatory signals, a T cell encountering an antigen undergoes abortive activation. Appreciable amounts of cytokine are not produced, division does not occur, and TCR expression is

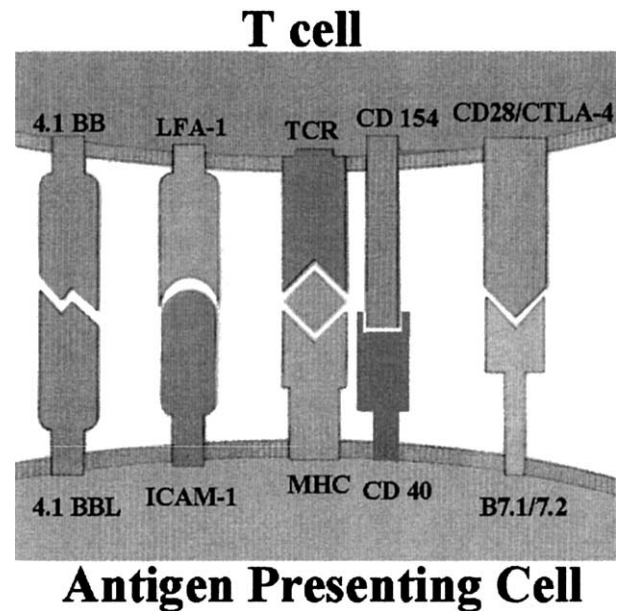


FIG. 2. Molecules involved in costimulation: Once the T cell receptor (TCR) recognizes a specific peptide lodged in the peptide binding groove of the major histocompatibility complex (MHC), a series of interactions follow, which lead to clonal expansion or deletion. Upregulation of CD154 on the T cell surface results in binding CD40 inducing expression of B7.1/7.2 on the APC. If the concentration of CD28 is sufficient to bind B7.1/7.1, costimulatory signals induce a clonal expansion of activated T cells. However, if CTLA-4 binds B7.1/7.2, an inhibitory signal will be delivered. Alternatively, 4.1BB may work as a substitute for CD28 delivering a costimulatory signal for T cell activation. The interaction between ICAM-1 and LFA-1 stabilizes the cell-cell interaction allowing the steric relationship necessary for optimal antigen presentation.

downregulated. Instead, it becomes anergic or undergoes apoptosis. In contrast, in the presence of costimulatory signals, T cells elaborate factors to promote development of an expanded population of mature effector cells capable of mediating a sustained immune response. (The costimulatory interactions are considered in greater detail below.)

CD40 and CD154

CD40 is a member of the TNF receptor family and is expressed on a variety of cells. CD154 is a glycoprotein member of the TNF family of molecules and is expressed predominantly on the surface of activated CD4⁺ T cells. The CD40-CD154 interaction is important for humoral immunity. It provides essential B cell survival signals, augments immunoglobulin production, and induces isotype switching. Antigen-specific ligation of the TCR with a peptide-MHC complex induces a transient upregulation of CD154 on the T cell surface. The T cell then activates the APC through the interaction of constitutively expressed CD40 on the APC. Engagement of CD40 induces expression of B7, CD44H and ICAM-1 molecules on APC cell surface. CD40 stimulation of macrophages and dendritic cells induces IL-12 production, which promotes interferon (IFN)- γ synthesis and proinflammatory immune responses [2, 8–11].

CD28 and B7

Engagement of CD40 with CD154 upregulates expression of B7-1 and B7-2 on the APC surface. Binding of these ligands with constitutively expressed CD28 on the T cell surface is required for T cell activation. CD28 is a member of the immunoglobulin gene superfamily. Two mechanisms serve to optimize CD28-mediated optimization of T cell responses. First, costimulation allows T cells to respond to low levels of TCR ligation, supporting T cell responses at low antigen concentrations. This is accomplished by augmenting cytokine protein synthesis by increased IL-2, IFN, and TNF gene transcription and mRNA half-lives. Second, CD28 supports sustained T cell responses by preventing anergy and apoptosis. If the T cell encounters antigen in the absence of CD28-B7 interaction, there is failure of T cell activation. These anergic T cells remain refractory to repeat antigen presentation in the presence of CD28. In addition, CD28 upregulates Bcl-x gene expression, whose protein endproduct serves as a potent survival factor for T cells and prevents apoptosis [2, 8, 12–16].

CTLA4 and B7

CTLA4 is upregulated on the T cell surface 48–72 h after activation by antigen binding and CD40-CD154 interaction. It binds to B7 with a 10- to 100-fold greater

affinity than does CD28. Its precise function is as yet unknown. However, many pieces of evidence suggest that CTLA4 downregulates activated T cells [8, 17]. In the case of resting T cells initially induced to enter the cell cycle, CTLA4 ligation blocks expression of IL-2 and its receptor, leading to growth arrest. In the case of activated T cells, CTLA4 activation may lead to apoptosis. In CTLA4 knockout mice, a severe and fatal lymphoproliferative disease develops early in life. Administration of a CTLA4-Ig fusion protein, which blocks B7 interaction with CD28, prevents this disease. Conversely, withdrawal of CTLA4-Ig therapy leads to development of the lymphoproliferative process with massive lymphoid expansion and infiltration of critical organs, such as the heart. It is also clear that the negative regulatory role of CTLA4 is not dependent solely upon inhibition of CD28-mediated signaling. Still, other investigators have suggested that ligation of CTLA4 may also deliver a signal necessary for tolerance induction [2, 8, 17–20].

4-1BB and 4-1BB Ligand

Similar to CD154, 4-1BB (CD137) is a member of the TNF receptor gene family and is present on activated T cells. Its 4-1BBL ligand is present on activated APC. It has been shown that 4-1BBL can replace CD28 and provide T cell supportive signaling. It is possible that the 4-1BB system serves as a back-up, redundant system used in the absence of CD28 signalling [8, 21–23].

The Effector Arm of Acute Rejection

The cell population that is the most important in initiating rejection is the CD4⁺ T cell [24]. CD4⁺ cells can both initiate and mediate allograft rejection, whereas CD8⁺ T cells are primarily mediators of graft destruction. There are at least 2 major mechanisms by which T cells can deliver a lethal hit through cell-cell contact: 1) interaction of Fas ligand on the activated cytotoxic T cell with Fas expressed on the target cell, and 2) delivery of granzymes. There are a number of key features: 1) antigen specificity, 2) requirement for cell-cell contact, and 3) ability to destroy multiple cells in the absence of self-destruction [2].

In the presence of IL-2 and other stimulatory lymphokines, activated T cells proliferate and differentiate. Over the course of three to five days, direct graft destruction occurs. The Fas-FasL pathway is involved in the killing mediated by cytotoxic T cells, as well as clonal selection and control of lymphocyte activation [25–27]. Cytolysis also occurs through *de novo* synthesis of granzyme, a specific cytotoxic T cell associated serine protease, and perforin, a protein resembling complement 9. Granzymes are thought to regulate cytolytic specific factors, whereas perforin directly causes pores in the target cell. These pores result in osmotic swelling and cell death. IL-4, -5, and -6 induce B cells

to differentiate, proliferate, and produce donor specific antibody. These antibodies bind to target tissues and lead to graft destruction either through the direct activation of complement and/or by targeting antibody-dependent cell-mediated cytotoxicity. No similar antigen-specific cytotoxic mechanisms have been identified for macrophages or granulocytes. However, these cells elaborate soluble factors that potentiate the inflammatory response [2, 5, 24, 28].

CURRENT RATIONALE FOR IMMUNOSUPPRESSION

The majority of available immunosuppressive agents target T cells. Cyclosporine and tacrolimus prevent calcineurin activation and T cell production of IL-2, which is critical for proliferation [6]. Rapamycin blocks cell cycle-specific signal transduction through the IL-2 receptor [6]. Mycophenolate acts to inhibit T cell proliferation through inhibition of purine synthesis [4]. The development of these drugs has led in significant improvements in both patient and graft survival in the past decade. However, these agents are nonspecific and expose patients to the risks of opportunistic infection, nephrotoxicity, diabetes and neoplasia [2, 6]. In addition, these agents suppress the immune reactivity but do not promote tolerance. As a result, these immunosuppressive agents must be continually administered to transplant recipients. Ironically, the state of tolerance must be maintained by immune regulatory mechanisms that actively suppress damage to donor cells [2, 6, 8]. Although it prevents acute rejection, global immunosuppression also inhibits regulatory mechanisms required for induction and maintenance of tolerance. Theoretically, the use of generalized immunosuppression would therefore preclude induction of tolerance.

HISTORICAL BACKGROUND TO TOLERANCE RESEARCH

The first record of successful transplantation appears in the third-century legend of Saints Cosmas and Damian. According to legend, these physician brothers used the limb of a Moor who had died to replace the cancerous leg of a church leader. A Fra Angelico painting has immortalized the miracle of the saints' patient who walked about with one white and one black leg [29]. In 1954, Merrill et al. performed the first successful human vascular organ transplant [29]. A kidney transplant was performed between a monozygotic donor-recipient pair, with the genetic identity eliminating the need for immunosuppression. This renal graft survived until the death of the recipient 7 years later from heart disease.

The idea that specific immune unresponsiveness might reflect an acquired biological state was introduced by Owen in 1945 [31]. He observed that dizygotic twins of cattle had hematopoietic cells from both twins

in the circulation, presumably the result of exchange of hematopoietic stem cells. This was especially striking given the immunogenicity of hematopoietic precursors by the twin fetuses. Subsequently in 1953, Billingham and coworkers induced hematopoietic chimerism in fetal and newborn animals [32]. They showed that tolerance induced by chimerism allows permanent engraftment of skin from the hematopoietic donor. In 1977, Wood and Monaco described a technique based upon administration of antithymocyte globulin and donor bone marrow cells to induce tolerance in skin grafted mice [33]. Thomas and colleagues then used the same approach to induce tolerance in a renal allograft model using rhesus monkeys [34, 35]. It had been recognized in the 1970s that blood transfusion before kidney transplantation improved graft survival [36]. The underlying mechanism was thought to be active induction of immune unresponsiveness through immune deviation or veto cell activity. Since then, a great deal of energy has been focused upon donor-specific transfusion (DST) mediated hyporesponsiveness [2]. Clinical use of DST has largely disappeared with the newer immunosuppressive agents and evidence for donor sensitization after DST.

The goal of achieving tolerance has long been the holy grail for transplant immunology. However, recently available reagents and a focus upon costimulatory pathways have reinvigorated clinical interest in the potential for inducing transplant tolerance.

CURRENT STATE OF TOLERANCE RESEARCH

The major scientific approaches to immune tolerance center upon the following strategies: 1) co-stimulatory blockade (e.g., anti-CD40 ligand, anti-B7, CTLA4-Ig), 2) cytokine modulation, 3) deletion of responding lymphocytes (e.g., Fas-ligand), and 4) other approaches, such as leukocyte migration blockade, peptide-based therapies targeting specific antigens, and use of molecularly engineered cells and tissues to inactivate pathogenic lymphocytes. These will be individually reviewed with an emphasis upon large animal and non-human primate animal models. *In vitro* studies and rodent model experiments will not be extensively reviewed. It should be noted that most experimental protocols use prolonged allograft survival in the absence of immunosuppression as an endpoint. This is necessitated by the requirement for lifelong graft observation to fulfill the theoretical definition of tolerance.

Costimulatory Blockade

As previously described, optimal proliferation of T cells with subsequent cytokine release requires the engagement of additional costimulatory molecules on T cells. Blocking the interaction between specific costimulatory molecules and their ligands can induce a

state of anergy or unresponsiveness. As a result, specific regimens have been developed which are designed to induce T cell anergy through co-stimulatory blockade. We will focus upon the following co-stimulatory molecule combinations: CD4-MHC class II, CD28-B7, and CD40-CD154 (also known as CD40 ligand).

CD4 binds MHC class II molecules on the surface of APCs. Antigen administered in combination with anti-CD4 Mab can induce tolerance to that antigen [37]. The underlying mechanism has not been precisely defined. It has been suggested that suppressor cell activity is induced (also termed infectious tolerance), implying that cells of the tolerant host can inhibit the response of normal T cells to the foreign graft [38, 39]. Others have suggested that anergy induction might also play a role; this hypothesis is based upon the observation that the tolerant state can be disrupted by addition of IL-2 [40, 41]. Alternatively, anti-CD4 Mab can induce Fas-mediated apoptosis of CD4+ T cells [42].

Despite the lack of a clear mechanism, it is apparent that anti-CD4 Mab therapy can induce long-term survival of islet and heart allografts in mice and rats [40, 43]. In a model of renal allograft survival in cynomolgus monkeys, administration of murine anti-human CD4 MAb significantly prolonged graft survival [44, 45]. Two isotypes were used a depleting IgG1 and a nondepleting IgG4; both prolonged graft survival. More recently, combination therapy using anti-CD4 polyclonal Ab with CTLA4-Ig, a fusion B7 antagonist protein, markedly prolonged cardiac allograft survival in HLA-mismatched monkeys [46]. Prolonged administration of the antibody maintained cardiac allograft survival until the antibody was cleared. The clinical applicability of this anti-CD4 based strategy is as yet unclear.

CD28 binds to B7, whereas CD154 binds to CD40. CD28 and CD154 appear to functionally interact in that engagement of one molecule augments the function of the other [8]. The positive feedback interaction between the two molecules enhances TCR signaling with enhanced proliferation and cytokine release. Recognition that costimulation signals are required for full T cell activation after TCR ligation has lead to several tolerance induction strategies based upon blockade of this interaction. The fusion protein CTLA4-Ig blocks co-stimulation via CD28 by binding to B& on APCs and has been demonstrated to significantly prolong kidney, heart and islet allograft survival in rodents [2, 47, 48]. It has been hypothesized that tolerance induction may result from selective inhibition of Th-1 type immune responses [8].

Combination immunomodulatory therapy with CTLA4-Ig and anti-CD154 Mab has been utilized in rodent models [49]. This strategy is based upon the hypothesis that anti-CD154 Mab will prevent CD40 interaction and associated coactivation, while simultaneously, CTLA4-Ig will prevent coactivation of allo-

reactive T cells that were either preactivated or escaped CD40-CD154 blockade. This combination therapy prolongs the survival of fully allogeneic skin, vascularized heart and aortic allografts in rodent models [47].

A dramatic demonstration of the efficacy of co-stimulatory blockade in induction of tolerance in non-human primate models of transplantation was recently published. In this study, CTLA4-Ig was administered with or without humanized anti-CD154 (hu5C8) in a rhesus monkey model of kidney transplantation [50]. Hu5C8 monotherapy administered for the first 14 days postoperatively prolonged rejection free survival for up to 100 days. Rejection that developed in monkeys treated with hu5C8 could be reversed by repeated treatment with the same antibody. Combination therapy with hu5C8 and CTLA4-Ig did not provide improved outcomes over that associated with hu5C8 alone. Interestingly, lymphocytes from rejection-free animals responded vigorously to donor and third-party cells in mixed lymphocyte reactions. These results motivated further studies in which hu5C8 was given over a prolonged period of time [51]. In these studies, after 5–6 months of hu5C8 therapy, there was no evidence of rejection for as long as 10 months after discontinuation of treatment. It is thought that prolongation of allograft survival in this setting is the result of down regulation of T cell activation. In other primate models of transplantation, administration of CTLA4-Ig alone to monkeys with chemically induced diabetes and transplanted with pancreatic islets resulted in normoglycemia for an extended time period in 2 of 5 animals [52]. It is this availability of humanized CD154 Mab and the success with its use in primate models that have resurrected the current interest in clinical applicability of transplantation tolerance.

Additional interest has been expressed in targeting CD2, CD30 or 4-1BB, adhesion molecules such as ICAM and VLA and intracellular signaling pathways for T cell activation such as CD45 and ZAP-70. Antibodies to LFA and ICAM-1 have been shown to induce permanent heart allograft and prolonged skin allograft survival in mice [53, 54]. These antibodies are thought to inhibit migration of host immune cells into the graft. The CD45 family of transmembrane protein tyrosine phosphatases play a central role in T cell signalling [55]. Antibody to CD45RB can prolong renal and islet cell allografts in mice [56]. The results from these antibody studies suggest that they may play a role in combination methodologies for tolerance induction. To date, there have not been any data generated in non-human primate models.

Cytokine Modulation

The discovery of T helper lymphocyte subsets (Th1 and Th2) that differ in their cytokine secretion pat-

terns and effector functions has provided a model for the regulation of immune and inflammatory processes by cytokines [56]. Th1 lymphocytes produce IL-2, IFN- γ , and lymphotoxin, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. Th1-derived cytokines are postulated to promote allograft rejection by mediating delayed type hypersensitivity reactions, cytotoxic T cell generation, macrophage activation, and antibody production to facilitate antibody-dependent cytotoxicity. In contrast, Th2-derived cytokines protect against rejection by suppressing delayed type hypersensitivity, counteracting IFN actions on macrophages, and deviating antibody production toward IgE and noncytotoxic subclasses of IgG. Expression of Th-1 cytokines in an allograft is often associated with acute rejection, whereas Th-2 expression correlates with graft acceptance [36]. Thus, the current Th-1 and Th-2 paradigm indicates that cytokines that promote inflammatory conditions, such as IFN- β and TNF, will promote graft rejection and T cell-mediated autoimmune attacks. In contrast, those that are anti-inflammatory, such as IL-10 and tumor growth factor- β , will promote autoantibody production but may alleviate graft rejection. However, important considerations include systemic vs local delivery of cytokines, synergistic or antagonistic effects, and the potential for replacing 1 type of immune response for another that is still destructive. In addition, it is unknown whether cytokines detected at the site of graft acceptance or rejection are causally related to the ongoing immune response. These cytokine profiles may be the result of: 1) a tolerant state induced by a different mechanism, 2) contribute ancillary support for maintaining a tolerant state, or 3) arise independently as a consequence of the tolerant state.

Many studies argue vigorously against a primary role for cytokine-mediated immune deviation in graft survival. In multiple cases of tolerance to grafts, cytokine profiles are neither Th-1 or Th-2 in character. Attempts to skew the cytokine immune response and induce tolerance have largely failed. Administration or expression of IL-10 and IL-4, inhibition of Th-1 cytokines, and induction of Th-2 cytokines by IL-12 blockade have not induced tolerance [56]. The recent use of knockout models in mice has generated further observations. These studies indicate that IL-2, IL-4, and IFN may contribute to acute rejection, but none are critical for rejecting fully allogeneic grafts. If a cytokine-based immunosuppressive strategy is to be successful, these results suggest that multiple cytokines or a common pathway for cytokine action, such as IL-2 receptor γ -chain, should be targeted. As has been recently stated, "in no circumstances to date has allograft tolerance been achieved by specifically blocking the effects of a proinflammatory cytokine and/or enhancing the endogenous production or local/systemic

administration of a cytokine with immunoregulatory properties" [57]. The lack of consistency in attempts at tolerance induction by cytokine modulation may be the result of redundancy in the cytokine families and the pleiotropic nature of individual cytokines. These characteristics have dampened initial enthusiasm for cytokine manipulation as a viable approach to the induction of transplantation tolerance.

Lymphocyte Deletion

Deletion of specific lymphocyte subgroups is another strategy currently undergoing study. Therapies that target the TCR-CD3 complex have proven to successfully prolong graft survival. Although Mab therapy to CD3 can effectively prevent allograft rejection when used as induction therapy, it is short-lived with a multitude of potentially life-threatening complications. An alternative form of anti-CD3 directed therapy has been developed that uses a novel T cell depleting immunotoxin [58]. This molecule is constructed by conjugating a diphtheria toxin binding site mutant to a murine monoclonal antibody directed toward rhesus monkey CD3. This reagent has been used to induce prolonged survival of kidney and skin allografts in monkeys when given in combination with intrathymic injection of donor lymphocytes [59]. This protocol appears to down-regulate antidonor cytotoxic T lymphocyte activity while maintaining helper T cell and B cell function and reactivity to third-party allografts. However, 3 long-term limitations associated with the use of this agent are chronic rejection, interstitial nephritis, and an unexplained wasting disease [59].

Another route of investigation uses the Fas-Fas ligand pathway for activation-induced cell death [60]. Analysis of apoptotic pathways in lymphocytes has demonstrated an essential role for members of the Fas/TNF receptor superfamily in control of the immune response. Immunologically privileged tissues protect themselves from immune attack through expression of Fas ligand. Attempts have been made to engineer FasL into donor tissues and APCs to selectively kill any T cell that engages the donor cells. Allogeneic pancreatic islets were cotransplanted with syngeneic myoblasts genetically engineered to express FasL [61]. Composite grafting protected the islet grafts from immune rejection and maintained normoglycemia for more than 80 days in mice with streptozotocin-induced diabetes. The FasL signal provided site- and immune-specific protection of islet allografts. The potential of this approach is not yet established because of uncertainties associated with the proinflammatory effects of FasL and the effect of FasL engagement on donor cells. The role of apoptosis has also been demonstrated in another setting in which apoptotic depletion of alloreactive cells is required to reduce this population sufficiently to allow control through anergy or

immunoregulation [62]. As a result, MHC mismatched heart and skin allografts would be accepted. This entire Fas–FasL approach emphasizes the potential role of “negative” regulation in the induction of tolerance.

Chimerism

Donor specific cells are frequently detected in the lymphoid tissues of a solid organ allograft recipient. Such chimerism persists in some instances for long periods after transplantation and may play an active role in the induction and/or maintenance of tolerance. Therefore, another strategy attracting interest for the prevention of transplant rejection is through donor hematopoietic chimerism. The host hematopoietic system is partially ablated and replaced with that of the donor through bone marrow transplantation. This form of tolerance is thought to generate a secure and robust tolerant state. The historic observation based upon lymphohematopoietic chimerism, originally noted by Owen in freemartin cattle, has since been revisited in both rodents and primates to achieve transplantation tolerance [31].

Passenger leukocytes in the graft are thought to induce a state of microchimerism in the recipient. In these microchimeric graft recipients, donor tissue hyporesponsiveness has been documented to correlate with engraftment of donor cells within the transplanted tissue and graft survival [63, 64]. These donor-derived dendritic cells are hypothesized to emigrate from the donor graft and migrate to host lymphoid tissue to induce tolerance through clonal deletion. Groups have used donor bone marrow transplantation to achieve mixed chimerism and tolerance [65]. However, unlike that which develops after allotransplantation, donor bone marrow-derived chimerism requires T cell depletion of the recipient with partial or complete ablation of the recipient's lymphohematopoietic system.

Early studies used polyclonal anti-thymocyte globulins and/or myeloablative regimens, such as radiation, to remove most of the host T cells and create sufficient hematopoietic space for the new marrow to engraft. Subsequently, the availability of specific ablative and blocking Mab has allowed researchers to define the role of separate T cell subsets, quality and quantity of donor bone marrow, and degree of marrow myelosuppression required. In addition, these reagents allow sufficient stem cell engraftment for tolerance induction. Another attractive feature of this strategy is that tolerance has been achieved in preclinical models across the most stringent of histocompatibility barriers. This strategy is sufficiently attractive that it has been utilized in the preclinical setting. Donor bone marrow transplantation have been used as an adjunctive therapy to augment donor cell chimerism in animal models of kidney, liver, heart, and pancreas transplants [60]. Based upon

observations from murine models, a T cell depleting, nonmyeloablative conditioning regimen for induction of mixed chimerism and tolerance of renal allografts in MHC-mismatched cynomolgus monkeys has been developed. Of 13 animals in this protocol, 11 achieved multilineage chimerism and 9 recipients survived long term. The longest period of survival exceeded 5 years [66].

These results notwithstanding, the logistics for application in human transplantation remain quite demanding. Grafts would be ideally transplanted as soon as possible after removal from the donor, thus limiting the window for transplantation to a very short time frame. In addition, there is still a great deal of controversy as to whether microchimerism is essential for transplantation tolerance. It has been suggested that persistent chimerism is required only to achieve tolerance through central tolerance within the thymus. Therefore, persistent microchimerism may not be necessary for peripheral immunoregulation-induced tolerance. In this case, indirectly processed antigen is sufficient to maintain the dominance of regulatory T cells. Other investigators have offered an alternative explanation for the persistence of donor-origin cells in tolerized graft recipients [65, 67]. They have argued that tolerance following donor bone marrow administration is actually “high dose, activation-associated tolerance,” another form of clonal deletion. In this schema, graft reactive cells are strongly activated, and this strong activation leads to clonal deletion.

Other Strategies for Tolerance Induction

Peptide-based hyporesponsiveness has been used as another strategy for tolerance induction. This focuses upon the role of indirect antigen presentation in allorecognition because donor APC cannot present these peptides by the direct presentation pathway. Peptides of MHC class I and II have been used to successfully induce tolerance in rat models when administered alone or with cyclosporine [68].

Injection of soluble antigen and cellular alloantigen into the thymus for tolerance induction was initially demonstrated in the 1960s [69]. Since then, the paradigm of intrathymic injection of alloantigen, usually intact donor cells, with deletion of preexisting T cells has been used to induce tolerance in islets, skin, heart, liver and kidney transplant models in rodents [2]. The basic intrathymic injection protocol has been extended by the use of DNA injection into the thymus for tolerance induction [70]. Both clonal deletion and peripheral suppression have both been implicated as the underlying mechanism. However, this technique has not yet been reported to induce tolerance in larger animals or primates. In addition, the progressive thymic involution that occurs in humans with age poses a theoret-

ical obstacle to use of this technique in mature individuals.

Correlative Human Observations

The use of universal immunosuppression in current clinical practice virtually eliminates possibility for evidence of immunologic tolerance in human allograft recipients. It is clear that induction of immune tolerance requires an intact immune system. Currently available data generated by Starzl derives from human graft recipients who retained their grafts despite having discontinued immunosuppression [71]. He suggests that passenger leukocytes migrate after transplantation and produce a microchimeric state that is essential and possibly even sufficient for allograft maintenance. This hypothesis is as yet unproven. Data are otherwise lacking to prove the existence of immunologic tolerance in humans after vascularized allograft transplantation.

Potential Pitfalls

The potential side-effects of tolerance to an allograft requires consideration. Among these are specificity, durability, disease recurrence, and the question of chronic rejection. Ideally, tolerance is induced in a specific fashion to donor-specific tissue, leaving the host immune system intact to respond to danger unimpaired. The ability to achieve such a high degree of specificity may not be possible. As a result, compromises in the recipient's infection defense or immune surveillance may result. The durability of induced tolerance is unknown. The immune requirements to maintain tolerance and the factors that may break it are unknown. If the host must mount an immune response to infection or trauma, it is unclear whether tolerance will persist. Chronic rejection is the primary reason for the demise of the majority of grafts. The pathophysiology of this entity is unknown, and the impact of tolerance induction on the genesis of chronic rejection is also unclear. Finally, immune tolerance may also adversely impact the recurrence of the original disease process that resulted in the initial organ insult. This consideration is particularly relevant for autoimmune disease processes, such as lupus nephritis, diabetes, and autoimmune cardiomyopathy.

CONCLUSION

Advances in transplantation biology have resulted in vastly improved patient outcomes after transplant surgery. However, the Achilles heel remains that of generalized immunosuppression with its associated predisposition toward infection and neoplasia. Transplant tolerance remains the ultimate goal for the field. Recent advances in understanding the mechanisms of T cell activation and technology for development of the

appropriate reagents offer the potential for achieving tolerance in the near future. However, despite the enthusiasm that meets new discoveries in transplant immunology, it remains clear that the human immune system is a remarkably complex and redundant mechanism that may not allow circumventing of such a basic function as recognition of nonself. In addition, it is also unclear which of the many available techniques will be able to safely induce tolerance.

REFERENCES

1. Cecka, J. M. The UNOS scientific renal transplant registry. In J. M. Cecka and P. I. Terasaki (Eds.), *Clinical Transplants*. Los Angeles: UCLA Tissue Typing Laboratory, 1998. Pp 1-14.
2. Rossini, A. A., Greiner, D. L., and Mordes, J. P. Induction of immunologic tolerance for transplantation. *Physiol. Rev.* **79**: 99, 1999.
3. Abbas, A., Auchinsloss, H., Austen, K. F., Bluestone, J., Carpenter, C., Chess, L., Davie, J., Fathman, C. G., and Howard, M. NIAID plan for research on immune tolerance. www.niaid.gov/publications/immune, 1-24, 1999.
4. Starzl, T. E., and Zinkernagel, R. M. Transplantation tolerance from a historical perspective. *Nat. Rev. Immunol.* **1**: 233, 2001.
5. Krensky, A. The immunobiology of transplantation. In R. L. Jamison and R. Wilkinson (Eds.), *Nephrology*. London: Chapman and Hall, 1997. Pp 1051-1071.
6. Sayegh, M., and Turka, L. A. The role of T cell costimulatory activation pathways in transplant rejection. *N. Engl. J. Med.* **338**: 1813, 1998.
7. Hayry, P., Isoniemi, H., Yilmaz, S., Mennander, A., Lemstrom, K., Raisanen-Sokolowski, A., Koskinen, P., and Ustinov, J. Chronic allograft rejection. *Immunol. Rev.* **134**: 33, 1993.
8. Gudmundsdottir, H., and Turka, L. A. T cell costimulatory blockade: new therapies for transplant rejection. *J. Am. Soc. Nephrol.* **10**: 1, 1999.
9. Lane, P., Traunecker, A., Hubele, S., Inui, S., Lanzavecchia, A., and Gray, D. Activated human T cells express a ligand for the human B cell associated antigen CD40 which participates in T cell dependent activation of B lymphocytes. *Eur. J. Immunol.* **22**: 2573, 1992.
10. Noelle, R. J., Ledbetter, J. A., and Aruffo, A. CD40 and its ligand, an essential ligand-receptor pair for thymus dependent B cell activation. *Immunol. Today* **13**: 431, 1992.
11. Clark, L. B., Foy, T. M., and Noelle, R. J. CD40 and its ligand. *Adv. Immunol.* **63**: 43, 1996.
12. Foy, T. M., Aruffo, A., Bajorath, J., Buhlman, J. E., and Noelle, R. J. Immune regulation by CD40 and its ligand gp39. *Annu. Rev. Immunol.* **14**: 591, 1996.
13. Lafferty, K. J., Cooley, M. A., Woolnough, J., and Walker, K. Z. Thyroid allograft immunogenicity is reduced after a period in organ culture. *Science* **188**: 259, 1975.
14. Linsley, P. S., Brady, W., Grosmaire, L., Aruffo, A., Damle, N. K., and Ledbetter, J. A. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J. Exp. Med.* **173**: 721, 1991.
15. Linsley, P. S., Brady, W., Urnes, M., Grosmaire, L. S., Damle, N. K., and Ledbetter, J. A. CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med.* **174**: 561, 1991.
16. Schwartz, R. H. Costimulation of T lymphocytes: the role of CD28/CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* **71**: 1065, 1992.
17. Karandikar, N. J., Vanderlugt, C. L., Walunas, T. L., Miller,

- S. D., and Bluestone, J. CTLA-4: a negative regulator of auto-immune disease. *J. Exp. Med.* **184**: 783, 1996.
18. Krummel, M. F., and Allison, J. P. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J. Exp. Med.* **182**: 459, 1995.
 19. Thompson, C. B., and Allison, J. P. The emerging role of CTLA-4 as an immune attenuator. *Immunity* **7**: 445, 1997.
 20. Tivole, E. A., Schweitzer, A. N., and Sharpe, A. H. Costimulation and autoimmunity. *Curr. Opin. Immunol.* **8**: 822, 1996.
 21. Pollok, K. E., Kim, Y. J., Zhou, Z., Hurtado, J., Kim, K. K., Pickard, R. T., and Kwon, B. S. Inducible T cell antigen 4-1BB: Analysis of expression and function. *J. Immunol.* **150**: 771, 1993.
 22. Goodwin, R. G., Din, W. S., Davis-Smith, T., Anderson, D. M., Gimpel, S. D., Sato, T. A., and Maliszewski, C. R. Molecular cloning of a ligand for the inducible T cell gene 4-1BB. *Eur. J. Immunol.* **23**: 2631, 1993.
 23. DeBenedette, M. A., Shahinian, A., Mak, T. W., and Watts, T. H. Costimulation of CD28-T lymphocytes by 4-1BB ligand. *J. Immunol.* **158**: 551, 1997.
 24. Krieger, N. R., Yin, D. P., and Fathman, C. G. CD4+ but not CD8+ cells are essential for allojection. *J. Exp. Med.* **184**: 2013, 1996.
 25. Rouvier, E., Luciani, M. F., and Golstein, P. Fas involvement in Ca independent T cell mediated cytotoxicity. *J. Exp. Med.* **177**: 195, 1993.
 26. Mogil, R. J., Radvanyi, L., Gonzalez-Quintal, R., Miller, R., Mills, G., Theofilopoulos, A. N., and Green, D. R. Fas participates in peripheral T cell deletion and associated apoptosis in vivo. *Int. Immunol.* **7**: 1451, 1995.
 27. Nagata, S., and Golstein, P. The Fas death factor. *Science* **267**: 1449, 1995.
 28. Markees, T. G., Gordon, E. J., Phillips, N. E., Shultz, L. D., Noelle, R. J., Mordes, J. P., Greiner, D. L., and Rossini, A. A. Prolonged skin allo- and xenograft survival in C57BL/6 CD4 knockout mice treated with anti-CD154 and donor spleen cells. *Trans. Proc.* **31**: 884, 1999.
 29. Klein, J. *Natural History of the Major Histocompatibility Complex*. New York: Wiley, 1986. Pp 1-775.
 30. Merrill, J. P., Murray, J. E., Harrison, J. H., and Guild, W. R. Landmark article Jan 28, 1956: successful homotransplantation of the human kidney between identical twins. *JAMA* **251**: 2566, 1984.
 31. Owen, R. D. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* **102**: 400, 1945.
 32. Billingham, R. E., Brent, L., and Medawar, P. B. Acquired immunological tolerance. *Nature* **172**: 603, 1953.
 33. Wood, M. L., and Monaco, A. P. The effect of timing of skin grafts on subsequent survival in ALS-treated marrow infused mice. *Transplantation* **23**: 78, 1977.
 34. Thomas, J. M., Carver, F. M., Cunningham, P. R. G., Olson, L. C., and Thomas, F. T. Kidney allograft tolerance in primates without chronic immunosuppression-the role of veto cells. *Transplantation* **51**: 198, 1991.
 35. Thomas, J. M., Carver, F. M., Kasten-Jolly, J., Haisch, C. F., Rebellato, L. M., Gross, U., Vore, S. J., and Thomas, F. T. Further studies of veto activity in rhesus monkey bone marrow in relation to allograft tolerance and chimerism. *Transplantation* **57**: 101, 1994.
 36. Opelz, G. D., and Terasaki, P. I. Poor kidney transplant survival in recipients with frozen blood transfusions or no transfusions. *Lancet* **2**: 696, 1974.
 37. Sablinski, T. H. W., Tilney, N. L., and Kupiec-Weglinski, J. W. CD4 monoclonal antibodies in organ transplantation. *Transplantation* **52**: 579, 1991.
 38. Onodera, H., Lehmann, M., Akalin, E., Volk, H. D., Sayegh, M. H., and Kupiec-Weglinski, J. W. Induction of infectious tolerance to MHC incompatible cardiac allografts in CD4 monoclonal antibody treated sensitized rat recipients. *J. Immunol.* **157**: 1944, 1996.
 39. Qin, S., Cobbold, S. P., Pope, H., Elliott, J., Kioussis, D., Davies, J., and Waldmann, H. Infectious transplantation tolerance. *Science* **259**: 974, 1993.
 40. Alters, S. E., Shizuru, J. A., Ackerman, J., Grossman, D., Seydel, K. B., and Fathman, C. G. Anti CD4 mediates clonal anergy during transplantation tolerance. *J. Exp. Med.* **173**: 491, 1991.
 41. Charlton, B., Auchinsloss, H., and Fathman, C. G. Mechanisms of transplantation tolerance. *Annu. Rev. Immunol.* **12**: 734, 1994.
 42. Banda, N. K., Bernier, J., Kurahara, D. K., Kurrle, R., Haigwood, N., Sekaly, R. P., and Finkel, T. H. Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation induced apoptosis. *J. Exp. Med.* **176**: 1099, 1992.
 43. Lehmann, M., Kupiec-Weglinski, J. W., Risch, K., Hancock, W. W., Muller, A., Kuttler, B., Hahn, H. J., Brock, J. W., and Volk, H. D. Anti-CD4 monoclonal antibody induced allograft tolerance in rats despite persistence of donor reactive T cells. *Transplantation* **64**: 1181, 1997.
 44. Mourad, G. J., Preffer, F. I., Wee, S. L., Powelson, J. A., Kawai, T., Delmonico, F. L., Knowles, R. W., Cosimi, A. B., and Colvin, R. B. Humanized IgG1 and IgG4 anti CD4 monoclonal antibodies-effects on lymphocytes in the bloodlymph nodes and renal allografts in cynomolgus monkeys. *Transplantation* **65**: 632, 1998.
 45. Hamawy, M. M., and Knechtle, S. J. Strategies for tolerance induction in nonhuman primates. *Curr. Opin. Immunol.* **10**: 513, 1998.
 46. Krieger, N. R., Yuh, D., McIntyre, B., Flavin, T. F., Yin, D. P., Robbins, R., and Fathman, C. G. Prolongation of cardiac graft survival with anti-CD4 Ig plus hCTLA4Ig in primates. *J. Surg. Res.* **76**: 174, 1998.
 47. Linsley, P. S., Brady, W., Urnes, M., Grosmaire, L. S., Damle, N. K., and Ledbetter, J. A. CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med.* **174**: 561, 1991.
 48. Larsen, C. P., Alexander, D. Z., Hollenbaugh, D., Elwood, E. T., Ritchie, S. C., Aruffo, A., Hendrix, R., and Pearson, T. C. CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation* **61**: 4, 1996.
 49. Larsen, C. P., Elwood, E. T., Alexander, D. Z., Ritchie, S. C., Hendrix, R., Tucker-Burden, C., Cho, H. R., Aruffo, A., Hollenbaugh, D., Linsley, P. S., Winn, K. J., and Pearson, T. C. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* **381**: 434, 1996.
 50. Kirk, A. D., Harlan, D. M., Armstrong, N. N., Davis, T. A., Dong, Y., Gray, G. S., Hong, X., Thomas, D., Fechner, J. H. J., and Knechtle, S. J. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc. Natl. Acad. Sci. USA* **94**: 8789, 1997.
 51. Kirk, A. D., Burkly, L. C., Batty, D. S., Baumgartner, R. E., Berning, J. D., Buchanan, K., Fechner, J. H., Germond, R. L., and Kampen, R. L. Treatment with humanized monoclonal antibody against CD154 prevents acyete renal allograft rejection in nonhuman primates. *Nat. Med.* **5**: 686, 1999.
 52. Levisetti, M. G., Padrid, P. A., Szot, G. L., Mittal, N., Meehan, S. M., Wardrip, C. L., Gray, G. S., Bruce, D. S., Thistlewaite, R. J., and Bluestone, J. A. Immunosuppressive effects of human

- CTLA4Ig in a nonhuman primate model of allogeneic pancreatic islet transplantation. *J. Immunol.* **159**: 5187, 1997.
53. Isobe, M., Yagita, H., Okumura, K., and Ihara, A. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. *Science* **255**: 1125, 1992.
54. Iwata, T., Kamei, Y., Esaki, S., Takada, T., Torii, S., Yamashita, A., Tomida, S., Tamatani, T., Miyasaka, M., and Yoshikai, Y. Immunosuppression by anti-ICAM-1 and anti-LFA-1 monoclonal antibodies of free and vascularized skin allograft rejection. *Immunobiology* **195**: 160, 1996.
55. Chan, A. C., Desai, D. M., and Weiss, A. The role of protein tyrosine kinases and protein tyrosine phosphatases in T cell antigen receptor signal transduction. *Annu. Rev. Immunol.* **12**: 555, 1994.
56. Lakkis, F. G. Role of cytokines in transplantation tolerance: lessons learned from gene-knockout mice. *J. Am. Soc. Nephrol.* **9**: 1, 1998.
57. Nickerson, P., Steiger, J., Zheng, X. X., Steele, A. W., Steurer, W., and Strom, T. B. Manipulation of cytokine networks in transplantation: false hope or realistic opportunity for tolerance. *Transplantation* **63**: 489, 1997.
58. Neville, D. M., Scharff, J., Hu, H. Z., Rigaut, K., Shiloach, J., Slingerland, W., and Jonger, M. A new reagent for the induction of T cell depletion, anti-CD3-CRM9. *J. Immunother.* **19**: 85, 1996.
59. Knechtle, S. J., Vargo, D. J., Fechner, J., Zhai, Y., Wang, J., Hanaway, M. J., Scharff, J., Hu, H., Knapp, L., Watkins, D., and Necille, D. M. FN18-CRM19 immunotoxin promotes tolerance in primate renal allografts. *Transplantation* **63**: 1, 1997.
60. Waldmann, H. Transplantation tolerance- where do we stand? *Nature Med.* **5**: 1245, 1999.
61. Lau, H. T., Yu, M., Fontana, A., and Stoeckert, C. J. Prevention of islet allograft rejection with engineered myoblasts expressing FasL in mice. *Science* **273**: 109, 1996.
62. Zhang, H. G., Su, X., Liu, D., Liu, W., Yang, P., Wang, Z., Edwards, C. K., Bluethmann, H., Mountz, J. D., and Zhou, T. Induction of specific T cell tolerance by Fas ligand expressing antigen presenting cells. *J. Immunol.* **162**: 1423, 1999.
63. Reinsmoen, N. L., Jackson, A., McSherry, C., Ninova, D., Wiesner, R. H., Kondo, M., Krom, R. A., Hertz, M. I., Bolman, R. M., and Matas, A. J. Organ-specific patterns of donor antigen specific hyporeactivity and peripheral blood allogeneic microchimerism in lung, kidney and liver transplant recipients. *Transplantation* **60**: 1546, 1995.
64. Reinsmoen, N. L., and Matas, A. J. Evidence that improved late renal transplant outcomes correlates with the development of in vitro donor antigen specific hyporeactivity. *Transplantation* **55**: 1017, 1993.
65. Bishop, G. A., McCaughan, G. W., Sun, J., and Sheil, A. G. Microchimerism and transplant tolerance. *Immunol. Today* **18**: 455, 1997.
66. Kawai, T., Sachs, D. H., and Cosimi, A. B. Tolerance to vascularized organ allografts in large animal models. *Curr. Opin. Immunol.* **11**: 516, 1999.
67. Webb, S., Morris, C., and Sprent, J. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* **63**: 1249, 1990.
68. Cuturi, M. C., Josien, R., Douillard, P., Pannetier, C., Cantarovich, D., Smit, H., Menoret, S., Pouletty, P., and Clayberger, C. Souillou: Prolongation of allogeneic heart graft survival in rats by administration of a peptide from the alpha 1 helix of the first domain of HLA-B7-01. *Transplantation* **59**: 661, 1995.
69. Vojtiskova, M., and Lengerova, A. Thymus-mediated tolerance to cellular antigens. *Transplantation* **6**: 13, 1968.
70. Knechtle, S. J., Wang, J., Graeb, C., Zhai, Y., Hong, X., Fechner, J. H., and Geissler, E. K. Direct MHC class I complementary DNA transfer to thymus induces donor specific unresponsiveness which involves multiple immunologic mechanisms. *J. Immunol.* **159**: 152, 1997.
71. Starzl, T. E., Demetris, A. J., Murase, N., Trucco, M., Thomson, A. W., and Rao, A. S. The lost chord: microchimerism and allograft survival. *Immunol. Today* **17**: 577, 1996.