Review Articles

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FRANKLIN H. EPSTEIN, M.D., *Editor*

THE ROLE OF T-CELL COSTIMULATORY ACTIVATION PATHWAYS IN TRANSPLANT REJECTION

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ORGAN transplantation is now common. For patients with end-stage cardiac, hepatic, or pulmonary failure, it can be lifesaving.[1] For patients with diabetes mellitus, pancreatic transplantation offers the chance of a cure and the arrest or reversal of diabetic complications such as neuropathy and retinopathy. In 1996 more than 19,410 organ and tissue transplantations were performed in the United States, and 53,755 patients were on waiting lists for transplantation.

Over the past two decades, the development of new immunosuppressive drugs with improved efficacy and decreased toxicity has led to substantial improvement in the survival of patients and in short-term graft survival for all organs. For example, before cyclosporine became available, the one-year survival of cadaveric renal allografts was approximately 65 percent, whereas it now exceeds 80 to 85 percent.[2] Much of this improvement can be attributed to better prophylaxis against and treatment for acute rejection, an immune response against the graft that usually occurs within the first six months after transplantation.

Despite the improvement in short-term results, the long-term survival of grafts that are functional at one year has changed little. For example, only 50 percent of cadaveric renal grafts that survive the first 12 months are still functioning 7.5 to 9.5 years later.[2] The cause of late graft loss is usually chronic rejection, a poorly understood disorder that may be mediated by both alloantigen-dependent and alloantigen-independent mechanisms.[3,4] Clinically, chronic rejection is manifested by gradually progressive graft dysfunction, leading ultimately to organ failure. In renal and cardiac allografts, the principal pathologic manifestation of chronic rejection is arteriosclerosis in the graft (Fig. 1); in lung transplants it is bronchiolitis obliterans. These lesions currently represent the most important factors limiting the long-term survival of transplanted organs.[3,4]

Several factors have been implicated in the development of chronic rejection. They include a chronic low-level host immune response to alloantigens expressed by the graft, early episodes of acute rejection (which still occur in up to 50 percent of graft recipients), and side effects of current medications (such as cyclosporine nephrotoxicity).[3,4] Therefore, improved methods of immunosuppression should have a substantial effect on long-term allograft survival.

Although many cells can participate in the process of acute transplant rejection, only T lymphocytes appear to be absolutely required.[5] Mice with genetic or induced T-cell deficiency cannot reject grafts because they lack the cellular mechanisms to recognize an antigen as foreign. The development of an effective immune response requires that T cells be activated by antigen and a second, costimulatory signal.[6] In a recent review in the *Journal,* Reiser and Stadecker[7] summarized the role of the B7 T-cell costimulatory pathway in infectious and autoimmune diseases. In this article, we will review the role of T cells and T-cell costimulatory activation pathways in transplant rejection, focusing on B7 and the related CD40 pathway.

ROLE OF T CELLS IN TRANSPLANT REJECTION

Rejection of organ and tissue allografts occurs because the mammalian genome contains several polymorphic loci that encode widely expressed tissue antigens.[5] Persons who do not express a given allele at any of these loci recognize the protein encoded by that allele as foreign and mount a vigorous immune response that results in graft rejection. The most important genes are clustered within the major histocompatibility complex (MHC), which in humans is known as the HLA complex. MHC molecules are expressed on the surface of antigen-presenting cells such as macrophages and dendritic cells. Their normal function is to bind fragments of antigenic peptides derived from invading microorganisms and pre-

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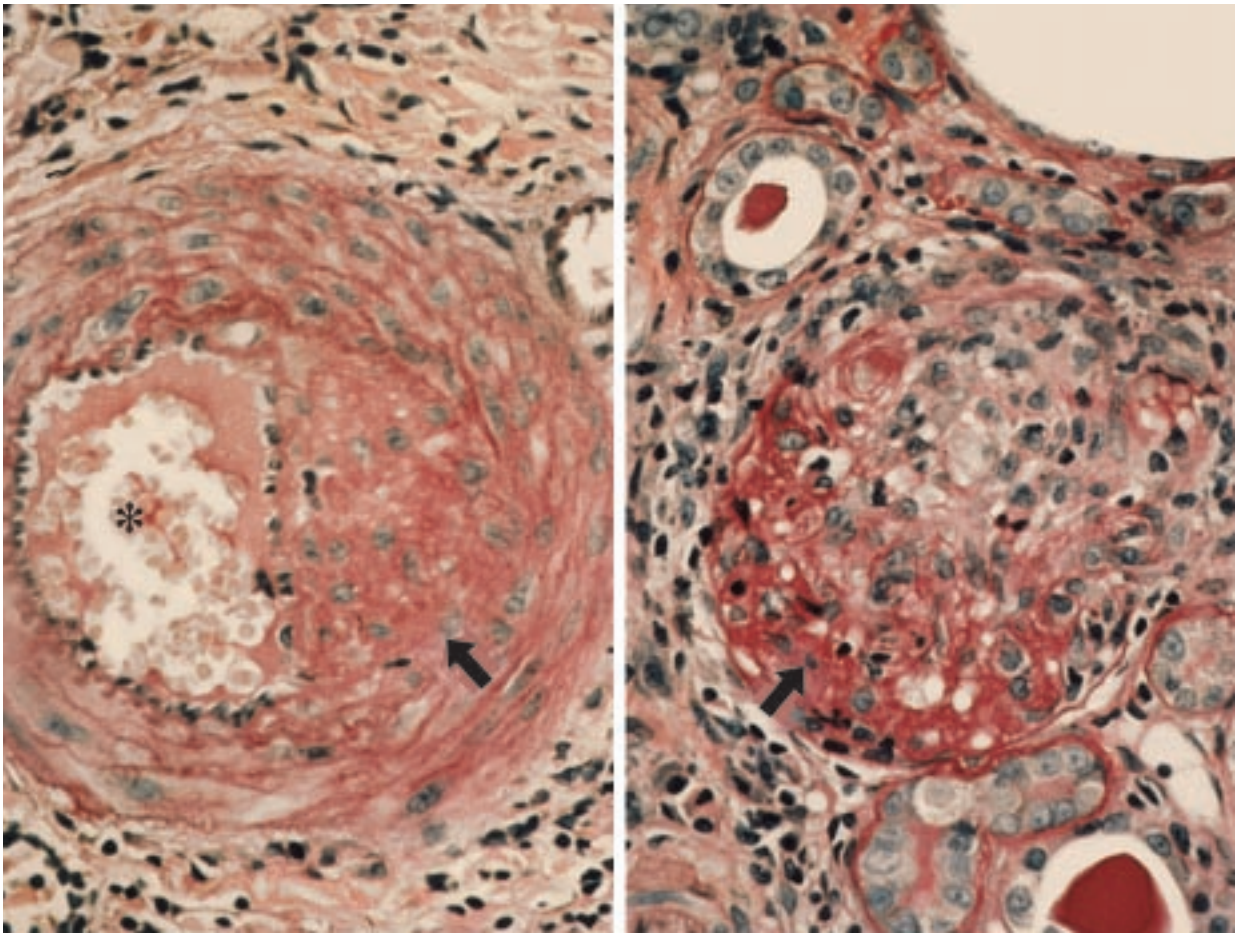

Figure 1. Histologic Appearance of Chronic Rejection in a Renal Allograft (×400).

A renal-biopsy specimen from a patient with chronic rejection shows a typical arterial lesion (left panel) and a typical glomerular lesion (right panel). This type of arterial lesion, also called graft arteriosclerosis, is similar to that seen in coronary vessels in heart-transplant recipients and is pathognomonic for chronic allograft rejection. It consists of intimal proliferation involving mostly smooth-muscle cells (arrow), leading to progressive luminal narrowing (asterisk) with resultant ischemia. The glomerular lesion consists of mesangial proliferation and occlusion of capillary spaces, leading ultimately to glomerulosclerosis (arrow). The net result of these two lesions is interstitial atrophy and fibrosis. (Photographs courtesy of Drs. N.L. Tilney and C.B. Carpenter, Brigham and Women's Hospital, Boston.)

sent them to T cells, which recognize them through their antigen receptors. This recognition step initiates T-cell–mediated immune responses.[5]

Transplanted organs express the MHC molecules of the donor, resulting in two pathways of antigen recognition (Fig. 2).[8] In the direct pathway, recipient T cells recognize allogeneic MHC (foreign MHC) molecules expressed on donor cells. Since T cells normally recognize foreign peptides bound to the body's own MHC (self MHC) molecules, it is thought that in the direct pathway, T cells recognize intact allogeneic MHC molecules because of molecular mimicry — that is, allogeneic MHC resembles self MHC plus foreign peptide at the three-dimensional level.[9] In the indirect pathway, peptides derived from the catabolism of donor MHC molecules are presented by self MHC on recipient antigen-presenting cells, in a fashion similar to the normal process for the presentation of bacterial or viral antigens. The direct pathway may be responsible for the vigorous immune response in acute rejection, whereas the indirect pathway may have the dominant role in chronic rejection.[10,11]

T-cells express either the CD4 or the CD8 glycoprotein on their surface.[5] CD4 cells, so-called helper T cells, are believed to be the most important for initiating graft rejection.[12] They are responsible for the production of most of the cytokines that are necessary to stimulate an immune response.[5] These cytokines act in an autocrine manner on the CD4 cells

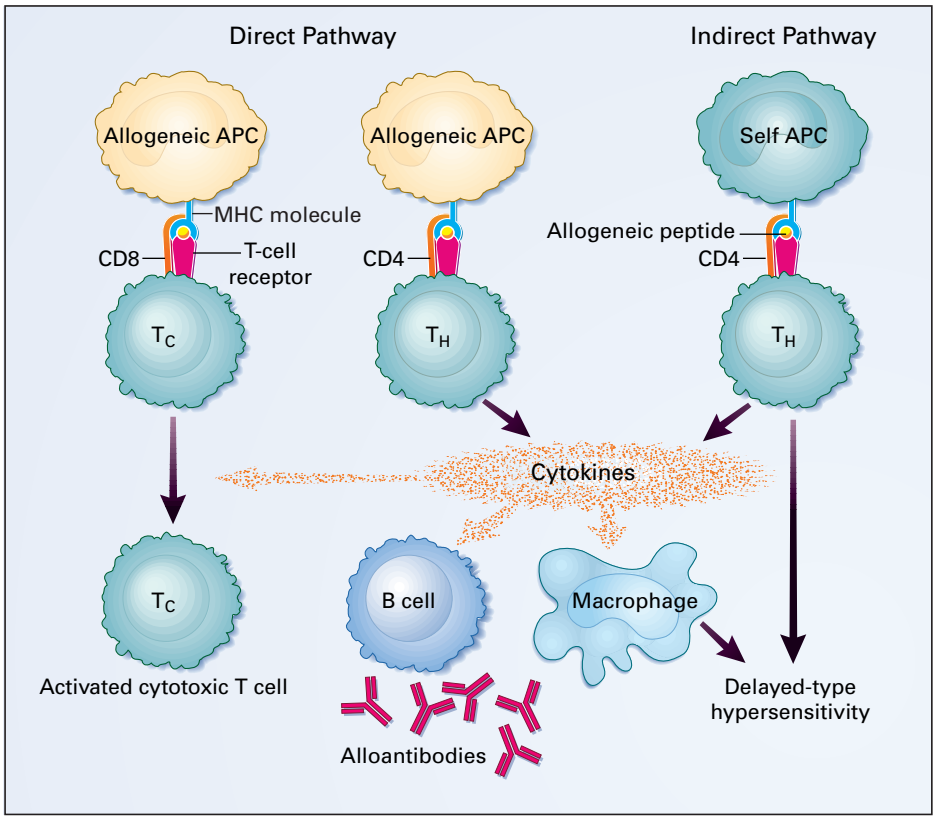

Figure 2. Pathways of Recognition of Allogeneic MHC Molecules and Mechanisms of Graft Rejection.
Graft rejection is usually initiated by CD4 helper T cells (T_H) that bind peptides in complexes with MHC class II molecules on antigen-presenting cells. In the direct pathway of recognition, an MHC molecule on a foreign (allogeneic) cell, such as an antigen-presenting cell (allogeneic APC), binds to the helper T cell. In the indirect pathway, the foreign MHC molecule is processed into peptides that are presented to the helper T cell by one of the body's own antigen-presenting cells (self APC). In either case, activated CD4 helper T cells proliferate and secrete a variety of cytokines that serve as growth and activation factors for CD8 cytotoxic T cells (T_C), B cells, and macrophages, which cause destruction of the graft by direct lysis of target cells, antibody production, and delayed-type hypersensitivity mechanisms, respectively.

themselves and in a paracrine manner on other cells, such as CD8 cells (most of which are cytotoxic T lymphocytes), macrophages, and B cells. Although CD8 T cells can produce small amounts of cytokines, their chief contribution to graft rejection is through direct lysis of donor cells. Both activated macrophages and CD4 T cells themselves contribute to graft rejection by a delayed-type hypersensitivity response involving the elaboration of soluble mediators such as tumor necrosis factor and reactive oxygen intermediates. This response is sufficient to cause graft loss, even in the absence of CD8 cells.[12] Although B cells do not directly participate in acute rejection when the recipient has not previously been exposed to the alloantigens in question, antibodies produced after such sensitization (e.g., by blood transfusion or previous transplantation) are the cause of hyperacute and accelerated rejection. Both delayed-type hypersensitivity reactions and alloantibodies may have a role in the process of chronic rejection.

CURRENT CONCEPTS IN IMMUNOSUPPRESSION

In keeping with the primary role of T cells in graft rejection, most immunosuppressive drugs currently in use or development target T cells.[13] The most successful drugs act in the early phases of T-cell activation. For example, cyclosporine and tacrolimus (formerly known as FK 506) each prevent T cells from producing cytokines critical for T-cell proliferation, such as interleukin-2.[14] Sirolimus (rapamycin), a promising drug in phase 3 clinical trials, blocks signal transduction by the interleukin-2 receptor.[15] Azathioprine and mycophenolate, both inhibitors of purine synthesis, also inhibit T-cell proliferation.[16] Monoclonal antibodies directed at T cells, such as OKT3 (muromonab-CD3), act by depleting T cells and blocking the T-cell antigen receptor.[17]

Although these drugs have led to considerable improvement in survival rates for both patients and grafts, there are at least two major problems associ-

ated with their use. First, immunosuppression with these drugs is nonspecific. Patients taking them have a substantial risk of opportunistic infections and cancer. There are also side effects not associated with their immunosuppressive actions, such as the nephrotoxic effects of cyclosporine and tacrolimus and the diabetogenic effects of tacrolimus.

Second, these drugs suppress the immune response to the allograft but do little to induce immunologic tolerance toward the graft. A tolerant state is usually defined as one in which the host does not mount an immune response against an antigen. One consequence of the inability to induce tolerance is that patients must continue to receive these drugs for life. The tolerant state is often associated with, and may actually be maintained by, regulatory mechanisms that actively suppress immune damage to donor cells.[18] To this extent, global immunosuppression, while it prevents acute graft rejection, may also inhibit regulatory mechanisms important in the maintenance of tolerance. Ongoing subclinical graft injury, occurring as a result of the failure to induce tolerance, may ultimately lead to chronic rejection and graft loss. New immunomodulatory strategies that exploit normal mechanisms of tolerance to self-antigens, such as the need for costimulatory signals in T-cell activation, may permit the development of immunologic tolerance.

THE ROLE OF COSTIMULATORY SIGNALS IN T-CELL ACTIVATION

Full activation of T cells requires two distinct but synergistic signals.[6] The first signal, delivered through the T-cell antigen receptor, is provided by antigen itself and is responsible for the specificity of the immune response. The second, or costimulatory, signal is not antigen-specific. Many T-cell molecules may serve as receptors for costimulatory signals; the CD28 molecule is the best characterized of these molecules.[19,20] CD28 has two known ligands, B7-1 (CD80) and B7-2 (CD86), both of which are expressed primarily on activated antigen-presenting cells (Fig. 3). T cells also express CTLA-4, a molecule structurally similar to CD28 that also binds B7-1 and B7-2.[21] However, unlike CD28, CTLA-4 transmits an inhibitory signal that serves to terminate the immune response.[22]

One point that deserves emphasis is the critical role of costimulation in T-cell responses. In the absence of costimulatory signals, a T cell encountering an antigen undergoes abortive activation. It does not produce appreciable amounts of cytokines and does not divide, but instead becomes unresponsive to appropriate stimulation (anergic) for up to several weeks[23] or undergoes programmed cell death (apoptosis).[24] In contrast, T cells that receive costimulatory signals produce interleukin-2 and other cytokines, thus permitting the development of an expanded population of mature effector cells (T cells, B cells, and macrophages) capable of mediating a sustained immune response.

Two lines of evidence highlight the role of the B7:CD28 pathway of T-cell costimulation in transplant rejection. First, drugs that block the interactions between B7 and CD28 molecules can induce long-lasting nonresponsiveness of T cells to alloantigens in vitro.[25] Second, B7 molecules are induced on endothelium within 24 hours after the transplantation of vascularized organs.[26] This means that T cells can receive costimulatory signals throughout the transplanted organ itself. Such induction does not occur in transplants in which the donor and recipient are genetically identical, a finding that indicates that the expression of B7 occurs specifically as a result of allogeneic stimulation.

Recently, the CD40:CD40 ligand pathway, initially described as having a role in B-cell activation, has been recognized as a key pathway for T-cell activation as well (Fig. 4).[27] CD40 is expressed on antigen-presenting cells, such as B cells, macrophages, and dendritic cells, as well as other cell types, such as endothelial cells (Fig. 5).[28] The ligand for CD40 (originally called CD40 ligand and recently named CD154) is expressed on activated CD4 T cells. Stimulation of CD40 provides important signals for antibody production by B cells and strongly induces B7 expression on all antigen-presenting cells.[26,29] In this manner, the CD40:CD40 ligand system may have an important role in T-cell costimulation. Activation of antigen-presenting cells through CD40 also induces the expression of adhesion molecules and inflammatory cytokines that participate in T-cell activation.[30] Finally, the CD40:CD40 ligand pathway is an attractive target for transplantation immunology, since it is important for B-cell responses as well. Although alloantibodies do not have a major role in acute rejection, they are strongly implicated as a contributing cause of chronic rejection. Thus, strategies that inhibit both T-cell and B-cell responses may be particularly effective in prolonging both short-term and long-term allograft survival.

T-CELL COSTIMULATORY BLOCKADE IN TRANSPLANT REJECTION

Acute Rejection

A recombinant fusion protein consisting of the extracellular domain of CTLA-4 linked to the constant region of IgG1, known as CTLA-4–Ig, binds B7 molecules with higher affinity than does CD28 and therefore acts as a potent competitive inhibitor of B7:CD28–mediated T-cell costimulation.[21] Administration of CTLA-4–Ig proved to be useful in preventing an immune response to pancreatic islets transplanted from humans to mice[31] and prolonged the survival of cardiac allografts in rats.[32] However,

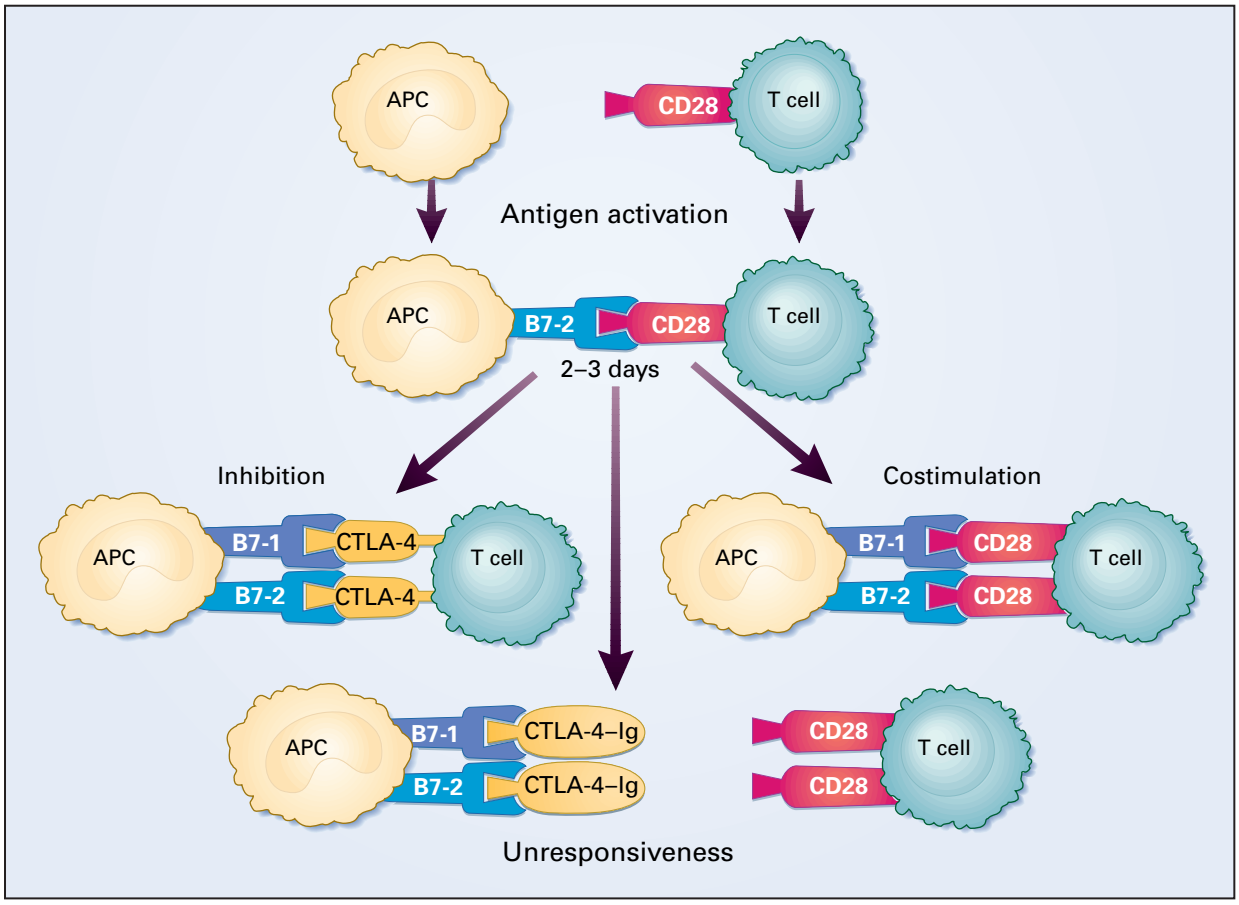

Figure 3. Functions of CD28, B7-1, B7-2, and CTLA-4 Molecules.

Resting T cells express CD28, but resting antigen-presenting cells (APCs) do not express B7 molecules. Within six hours after activation, B7-2 is expressed by antigen-presenting cells and is available to bind to CD28, transmitting a costimulatory signal to the T cell. By 48 to 72 hours after activation, antigen-presenting cells also express B7-1, whereas T cells express the CTLA-4 inhibitory receptor. Both B7-1 and B7-2 can bind to either CD28 or CTLA-4, providing continued costimulation or a new inhibitory signal, respectively. Because CTLA-4 binds B7 molecules with a higher affinity than does CD28, its inhibitory interaction eventually predominates, leading to the termination of the immune response. The fusion protein CTLA-4–Ig can compete with CD28 and CTLA-4 for B7 binding, thus preventing costimulatory interactions.

in the latter studies a true state of tolerance was not attained, and all grafts were ultimately rejected. A follow-up study indicated that the administration of donor splenocytes on the day of transplantation, followed by a single injection of CTLA-4–Ig on day 2 after transplantation, was effective in achieving permanent allograft survival in most animals.[33] The need for donor antigen in conjunction with CTLA-4–Ig for the induction of tolerance in cardiac transplantation has been confirmed.[34,35] It is likely that donor cells are required because cardiac tissue contains very few passenger leukocytes. These are bone marrow–derived cells, typically dendritic cells or monocytes, that express MHC class I and II molecules and migrate out of donor organs into regional lymph nodes shortly after transplantation. The mechanisms by which donor cells promote the induction of tolerance in these animal models are not known.

There have been many subsequent studies of transplantation in rats and mice, including studies of transplantation of islet, cardiac, renal, hepatic, lung, and skin allografts, in which the effects of blockade of the B7:CD28 pathway in vivo have been described. In virtually all these studies, blocking B7:CD28–dependent T-cell costimulation led to substantial prolongation of transplant survival, and in many instances donor-specific tolerance was achieved. From this work several interesting principles have emerged.

First, CTLA-4–Ig is most effective if its administration is delayed until after transplantation.[33,36] This may reflect the fact that cycling T cells are more susceptible to the absence of costimulatory signals than

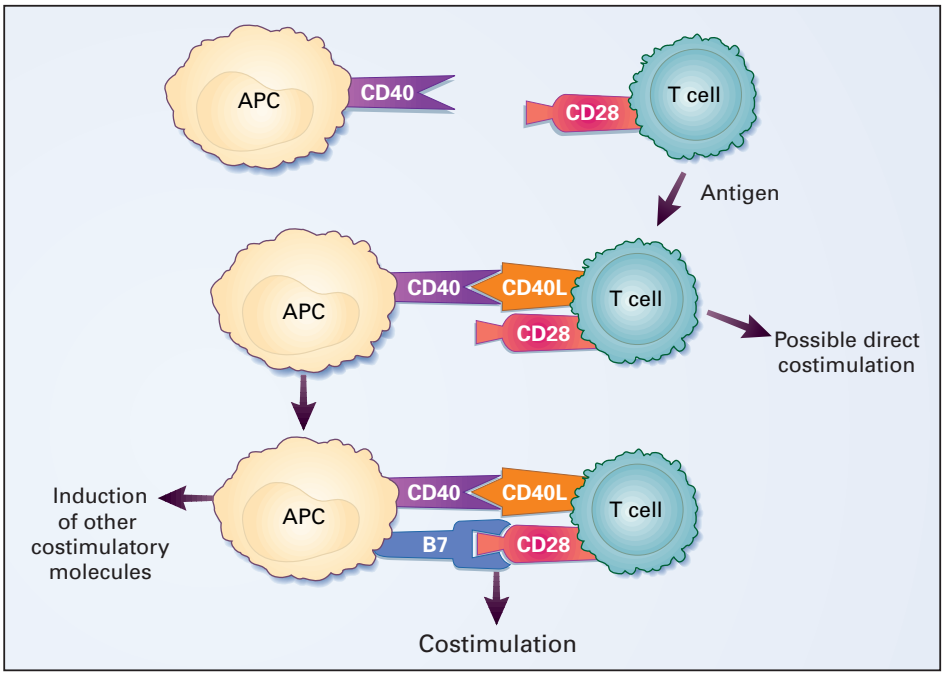

Figure 4. Relations between the CD28 and CD40 Ligand Pathways.

Resting antigen-presenting cells (APCs), which include B cells, macrophages, and dendritic cells, express CD40. When activated, T cells express CD40 ligand (CD40L). Interactions between CD40 ligand and CD40 are important in providing B-cell help to prevent apoptosis and induce immunoglobulin production and isotype switching. Activation of CD40 on APCs provides a signal for the induction of B7 molecules, particularly B7-2. CD40 ligand may act in T-cell costimulation by directly providing costimulation, by inducing B7, or by inducing other costimulatory ligands.

resting T cells. Furthermore, after activation, T cells are induced to express the CTLA-4 protein itself on their surface, which transduces a negative regulatory signal to the cell (Fig. 3).[22] Delaying the administration of CTLA-4–Ig would allow B7 molecules time to bind to CTLA-4, which may be required for the induction of tolerance.[37] However, if the administration of CTLA-4–Ig is delayed until the onset of acute rejection, it is ineffective in prolonging graft survival.[38]

Second, drugs that block either B7-1 or B7-2 alone do not appreciably prolong allograft survival.[39,40] This finding indicates that either B7-1 or B7-2 alone is sufficient to mediate graft rejection. In addition, since negative signals delivered through CTLA-4 can promote tolerance, blocking B7:CD28 costimulation without blocking the interaction between B7 and CTLA-4 may be the most effective way to block T-cell immune responses.[37]

Third, in some circumstances it may be possible to treat the allograft itself, thus obviating the need to administer drugs to the recipient. In one study, pretreatment of pancreatic islets with CTLA-4–Ig ex vivo before transplantation induced tolerance in almost 50 percent of recipients, even though the recipients themselves received no systemic therapy.[41]

Finally, the mechanism by which costimulatory blockade induces and maintains tolerance is not known. There is evidence for the induction of anergy, the deletion of antigen-reactive cells, and the induction of regulatory (i.e., suppressor) cells.

Chronic Rejection

In animal models, treatments that prevent acute rejection have often resulted in grafts that are said to be functioning at later points in time but that have histologic evidence of severe chronic rejection. There are at least two reasons for this. First, the time frame of the studies was short. Over a longer period, the grafts would probably have stopped functioning. Second, in many animal models the graft was placed as an adjunct to the native organ, not as a replacement. Therefore, the animal was not dependent on the function of the transplanted organ for survival. In human organ-transplant recipients, in whom function of the transplant is critical, this degree of chronic rejection and associated organ dysfunction may result in marked illness or death. Therefore, graft

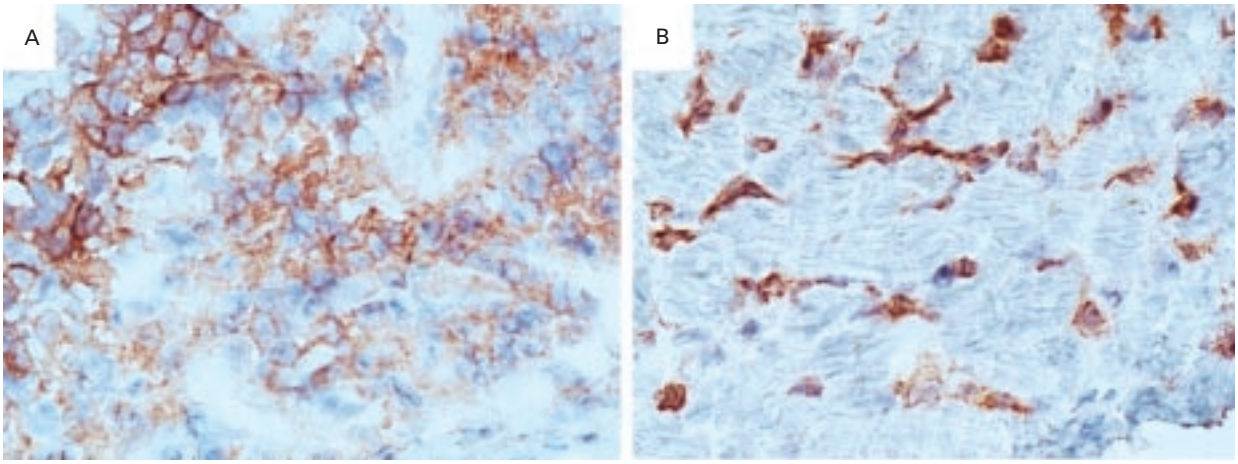

Figure 5. CD40 Expression during Human Cardiac-Allograft Rejection (×400).

Human cardiac-allograft tissue obtained during an episode of acute rejection occurring two to three months after transplantation was examined with immunoperoxidase staining for CD40. Panel A shows staining of CD40 (brown material) on infiltrating mononuclear cells, and Panel B shows CD40 on endothelial cells. (Photographs courtesy of Dr. David M. Briscoe, Children's Hospital, Boston.)

survival alone is not an adequate end point for use in assessing drug efficacy.

The pathophysiology of chronic allograft rejection is much less well understood than that of acute rejection. It is clear that alloantigen-dependent mechanisms initiate and are important in the progression of chronic rejection.[3] However, alloantigen-independent mechanisms such as graft ischemia and viral infections undoubtedly contribute to chronic allograft dysfunction.[4] Indeed, there may be a final common immunologic pathway of action for these events, because many antigen-independent factors increase the incidence of acute rejection, itself a known risk factor for the development of chronic rejection.

These considerations suggest that blocking T-cell responses in a manner that induces donor-specific tolerance should inhibit the development of chronic rejection. Indeed, in rats with cardiac transplants, blocking B7:CD28 interactions with a single dose of CTLA-4–Ig prolonged graft survival and prevented the development of graft arteriosclerosis, the characteristic finding of chronic rejection in cardiac allografts.[42] The results were similar in rats with renal allografts, in which progressive proteinuria serves as an indication of chronic rejection and graft dysfunction.[43] In contrast, in animals treated with cyclosporine in doses similar to those used in humans, chronic rejection and graft arteriosclerosis developed.

When animals treated with CTLA-4–Ig were given low-dose cyclosporine for the first month after transplantation, long-term graft survival was not affected, but severe arteriosclerosis developed, indicating that the protective effects of CTLA-4–Ig against the development of chronic rejection were abrogated by cyclosporine.[44] Graft arteriosclerosis did not develop in these animals when they were given donor cells in addition to cyclosporine and CTLA-4–Ig.[44] Although the protective mechanisms of donor cells are not known, these data have important implications for clinical strategies aimed at costimulatory blockade, because cyclosporine is currently being widely used in maintenance immunosuppressive regimens after transplantation. Blockade of the B7:CD28 costimulatory pathway prevented cold ischemia–reperfusion injury in rats with renal transplants.[45] These studies have particular clinical applicability in the transplantation of cadaveric renal grafts, an area in which initial ischemic injury has an important role in early graft dysfunction and appears to be a major alloantigen-independent risk factor for the development of chronic rejection and late graft loss.[4]

Blockade of the CD40:CD40 Ligand Pathway

Blocking the CD40:CD40 ligand costimulatory pathway by means of a monoclonal antibody against murine CD40 ligand is effective in preventing acute cardiac-allograft rejection in mice.[46] The effects of administered donor cells are synergistic with those of CD40 ligand blockade in preventing acute rejection and inducing long-term graft survival in mice with pancreatic-islet or cardiac transplants.[26,47]

Preliminary Studies of Transplantation in Large Animals

Treating rhesus monkeys that had received renal transplants with CTLA-4–Ig revealed possible synergy between CTLA-4–Ig and cyclosporine in delaying acute rejection and prolonging graft survival.[48]

CTLA-4–Ig has also been used to delay rejection of islet allografts in monkeys.[49] In nonhuman primates with cardiac transplants, blockade of CD40 ligand with a humanized monoclonal antibody was effective in delaying acute rejection.[50] Most recently, the combined administration of CTLA-4–Ig and anti–CD40 ligand monoclonal antibody induced long-term rejection-free renal-allograft survival in nonhuman primates.[51] This study suggested that the combination of CTLA-4–Ig and anti–CD40 ligand was more effective than either treatment alone, confirming earlier studies in mice.[52] There are no published data on the use of either CTLA-4–Ig or anti–CD40 ligand in human transplantation.

CONCLUSIONS

Recent advances in our knowledge of T-cell activation have suggested that inhibiting T-cell costimulatory pathways may be an effective way to promote antigen-specific tolerance of transplants. Blockade of the CD28 and CD40 pathways has shown great promise in preventing transplant rejection in rodents. Since only a short course of treatment is required, it may be possible to induce donor-specific tolerance with an otherwise intact immune system. However, rodents are more easily made tolerant to transplantation antigens than are larger animals and humans. Therefore, the next hurdle to be crossed is further translation of these results into studies of nonhuman primates and ultimately into clinical studies.

Phase 1 trials of CTLA-4–Ig in psoriasis, a T-cell–mediated disorder, indicate that this agent can inhibit T-cell–mediated responses in humans.[53] Studies with drugs that block the CD40:CD40 ligand pathway are ongoing in patients with idiopathic thrombocytopenic purpura. There still remain many unresolved issues with respect to how best to use these drugs in clinical organ transplantation, such as timing of administration, length of the treatment period, and whether to use them concurrently or consecutively with cyclosporine. Nonetheless, they have generated great excitement as a result of their successful use in animals with transplants and animals with autoimmune disease. It seems quite likely that costimulatory blockade with drugs that block either the binding of cell-surface molecules[54,55] or intracellular signal transduction will eventually become a clinical reality in organ transplantation.

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