

Mechanisms of allergic diseases

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Costimulation blockade in autoimmunity and transplantation

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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

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List of Design Committee Members: *Author:* Flavio Vincenti, MD
Activity Objectives

1. To understand the importance of costimulatory pathways in T-cell activation in autoimmune disease and transplantation.

2. To learn how the pharmacologic targeting of this pathway is being used to develop new therapies.

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Signaling through the costimulation receptors is a critical pathway in the regulation of T-cell activation. The selective costimulation inhibitor abatacept (cytotoxic T lymphocyte-associated antigen 4—Ig) binds to CD80 and CD86 on antigen-presenting cells, blocking interaction with CD28 on T cells, and is approved for the treatment of moderate to severe rheumatoid arthritis. Belatacept (LEA29Y), currently enrolling phase III trials in renal transplantation, was rationally designed from abatacept to bind with more avidity to CD86, providing the more potent immunosuppressive properties required for immunosuppression in transplantation. This review describes the relevant preclinical studies and summarizes recent clinical findings on these 2 molecules in autoimmune diseases and organ transplantation. Although both inhibit the CD28 costimulatory pathway, they are tailored for specific disease states—abatacept

for autoimmune diseases and belatacept for transplantation. (*J Allergy Clin Immunol* 2008;121:299-306.)

Key words: *Costimulation, abatacept, belatacept, organ transplantation, autoimmune disease, rheumatoid arthritis*

The *costimulation* pathway has been one of the most exciting and intensively researched areas in basic immunology in the past quarter century. These efforts culminated in 2005 with the approval by the US Food and Drug Administration (FDA) of abatacept (*cytotoxic T lymphocyte-associated antigen 4 [CTLA4]—Ig*) for rheumatoid arthritis and the publication of the positive results of the first clinical trial of belatacept, a second-generation CTLA4-Ig, in renal transplantation (Fig 1).¹⁻⁶ Blockade of costimulation offers the advantage of selective inhibition of T-cell responses and has the potential of inducing *tolerance* to specific antigens in both autoimmunity and transplantation.⁷⁻¹⁰ In transplantation, costimulation blockade represents a new paradigm in immunosuppression, with biologic agents used as maintenance therapy, replacing current drugs that require frequent therapeutic drug monitoring and are associated with chronic toxicities.¹¹

T lymphocytes play a central role in the initiation and regulation of the adaptive immune response to antigen, whether foreign or native. Naive T cells require 2 signals for their full activation.¹ The first, Signal 1, is an antigen-specific signal provided by the T-cell receptor interacting with the MHC and antigenic peptide complex on the antigen-presenting cell (APC). The second, or costimulatory, signal is provided by the interactions between specific receptors on the T cell and their ligands on the APC (Fig 2).

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Terms in boldface and italics are defined in the glossary on page 300.

Abbreviations used

APC:	Antigen-presenting cell
CNI:	Calcineurin inhibitor
CTLA4:	Cytotoxic T lymphocyte-associated antigen 4
DMARD:	Disease-modifying anti-inflammatory drug
FDA:	US Food and Drug Administration
IL-2R:	IL-2 receptor
MMF:	Mycophenolate mofetil
PTLD:	Posttransplant lymphoproliferative disease
RA:	Rheumatoid arthritis

Following these 2 signals, a number of pathways are activated. They include the calcium-*calcineurin* pathway, the *RAS* mitogen-activated protein kinase pathway, and the subsequent activation of several transcription factors for a number of effector compounds, including the cytokine IL-2. IL-2 activates the target of *rapamycin* pathway, sometimes referred to as *Signal 3*. These events induce T-cell proliferation, generation of an effector, CD4⁺ T-cell pool (T_H), and the clonal expansion of activated CD8⁺ or cytotoxic T cells.¹² If the T cell does not receive a

costimulation signal because of blockade of this pathway, it becomes anergic and undergoes apoptosis.

Multiple costimulatory pathways are involved in T-cell regulation; these can either upregulate or downregulate T-cell activation.¹³ Perhaps the most critical, and certainly the best characterized, costimulatory interactions are between *CD40* and *CD154* of the TNF:TNF receptor family and between *CD28* and CD80 and CD86 in the B7 family.¹³ The CD40-CD154 pathway was initially described as critical for B-cell activation and differentiation, but it was subsequently reported to contribute to T-cell activation by upregulating the B7 family ligands CD80 and CD86 on APCs. CD154 is expressed on vascular endothelial cells, smooth muscle cells, and macrophages, suggesting an expanded role for the CD40-CD154 pathway during immunity.²

Prolonged allograft survival was demonstrated in nonhuman primates treated with anti-CD154 mAbs. The first clinical trial in recipients of primary renal allografts with hu5C8, a humanized anti-CD154, was launched with great anticipation that this regimen might result in operational tolerance. However, therapy with anti-CD154 caused thrombotic events leading to discontinuation of the trial as well as cessation of the clinical development of anti-CD154 in both transplantation and

GLOSSARY

CALCINEURIN: A calcium-dependent phosphatase that is activated in response to T-cell activation; dephosphorylates the transcription factor nuclear factor of activated T cells, allowing cytoplasmic escape, entrance into the cell nucleus, and activation of gene transcription (including the IL-2 gene).

CD28: Expressed on most T cells, provides a second signal for T-cell activation on binding to CD80/86, resulting in the activation of the PI-3 kinase-Ras-mitogen-activated protein kinase signaling pathway.

CD40: Present on mature B cells, signal transduction through CD40 causes upregulation of CD80/86, thus facilitating costimulation.

CD154: Also known as CD40 ligand, on activated T cells, required for IgM class switch. Mutations in CD40 ligand cause X-linked hyper-IgM syndrome with absence of germinal centers and IgG, IgA, and IgE.

COSTIMULATION: T-cell activation requires 2 signals: (1) through the T-cell receptor interacting with antigen-MHC, and (2) a second signal, for example, through CD28:CD80/86 or CD40:CD40L; T cells that do not receive a second signal become anergic.

CYTOKINE STORM: The release of multiple inflammatory mediators resulting in fever, pain, hypotension, and multiorgan failure; can cause systemic inflammatory response syndrome. Cytokine storm in patients treated with anti-CD28 was associated with increased TNF- α , IL-2, IL-6, IL-10, and IFN- γ levels and increased numbers of CD4⁺, CD8⁺ T cells, B cells, and natural killer cells.

CYTOTOXIC T LYMPHOCYTE-ASSOCIATED ANTIGEN 4 (CTLA4): Also known as CD152, expressed on activated T cells, member of the immunoglobulin superfamily, cytoplasmic tail contains an immunoreceptor tyrosine-based inhibitory motif; binds to B7 (B7-1 = CD80; B7-2 = CD86) and limits IL-2 production and the activation signal through CD28:CD80/86; CTLA4^{-/-} mice have lymphoproliferation.

IL-1: Primary source is monocytes/macrophages, has multiple proinflammatory effects including increasing vascular endothelial activation for leukocyte migration, production of cytokines (eg, IL-6, TNF- α), and acute phase reactants. Blockade of IL-1 is used for RA and autoinflammatory syndromes.

NUCLEAR FACTOR- κ B (NF- κ B): A family of proteins that contain a Rel DNA binding domain, form homodimers and heterodimers, and are inactivated when bound to I κ B proteins. Phosphorylation of I κ B proteins

by I κ B kinases allows release of NF- κ B from the cytoplasm; IL-1 β , TNF- α , and T-cell and B-cell activation all activate I κ B kinases.

POSTTRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD): Lymphoid proliferation, often B-cell and EBV-associated, occurring after organ transplantation, may be associated with suppression of cytotoxic T-cell function caused by immunosuppressive regimens; World Health Organization classification scheme includes 3 types: hyperplastic (early lesions, acute infectious mononucleosis), monomorphic (B, T, or other type of lymphoma), and polymorphic (monoclonal or polyclonal).

PSORIASIS VULGARIS: Affects 90% of patients with psoriasis, distinct papulosquamous red/pink plaque lesions with silver scale most commonly on the extensor surfaces; 50% of patients will have nail changes including pitting, onycholysis, and oil spots; 25% of patients can have associated seronegative inflammatory psoriatic arthritis. Lesions are infiltrated with CD4⁺, CD8⁺, CD45⁺RO (memory) cells; increased vascular endothelial growth factor expression is modulated by TNF- α .

RAPAMYCIN: Blocks lymphocyte proliferation by inhibiting IL-2 signaling; binds to mTOR/FRAP/RAFT1/RAPT1 to inhibit kinase activity.

RAS: A family of GTPase proteins (small G proteins) that activate the mitogen-activated protein kinase pathway in activated T cells to induce gene transcription of cell cycle-associated proteins and IL-2.

REJECTION: Hyperacute: occurs in the first 48 hours posttransplant, caused by antibody deposition and activation of complement leading to organ thrombosis. Acute: occurs 6 to 90 days posttransplant, T-cell mediated. Chronic: occurs >2 months posttransplant.

TNF- α : Also known as *cachectin*, monocytes/macrophages are primary source, effects similar to IL-1 including increased vascular activation, acute phase reactants, MHCII expression, cytotoxicity. TNF blockers include etanercept (Enbrel), infliximab (Remicade), and adalimumab (Humira), used in RA, psoriasis/psoriatic arthritis, and Crohn disease.

TOLERANCE: Depletion of autoreactive T and B cells in the thymus and bone marrow constitutes central tolerance; peripheral antigens can be expressed in the thymus under the control of AIRE, a transcription factor involved in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome. Autoreactive T cells are inactivated through anergy, deleted through apoptosis, or suppressed by regulatory T cells, all of which is termed *peripheral tolerance*.

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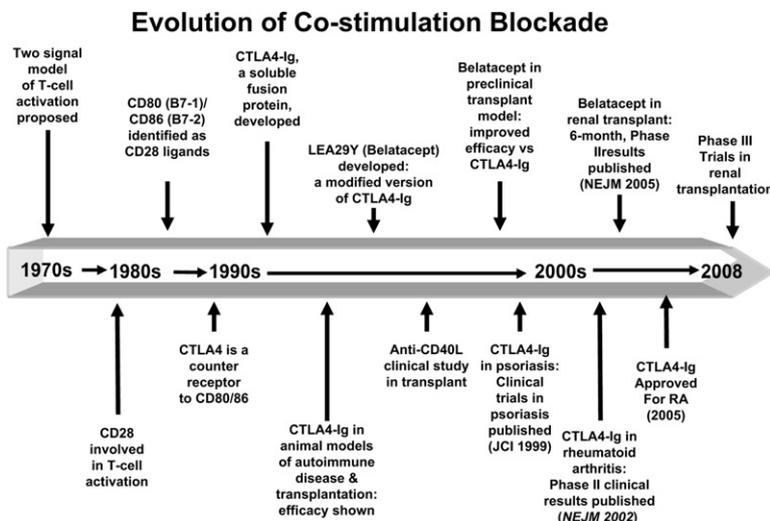


FIG 1. The evolution of the clinical development of costimulation blockade.

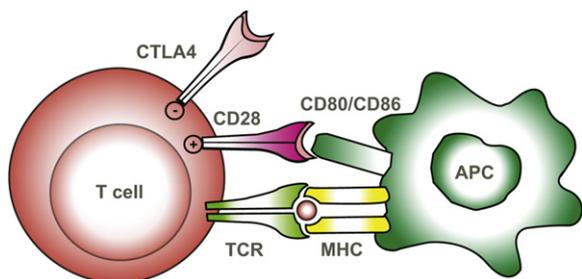


FIG 2. Two signals are required to activate the T-cell signal. One is antigen-driven, with the allopeptide in the groove of the MHC delivered to the T-cell receptor of the T cell. The second or costimulation signal is provided through the binding of the CD28 receptor with its ligands CD80 and CD86.

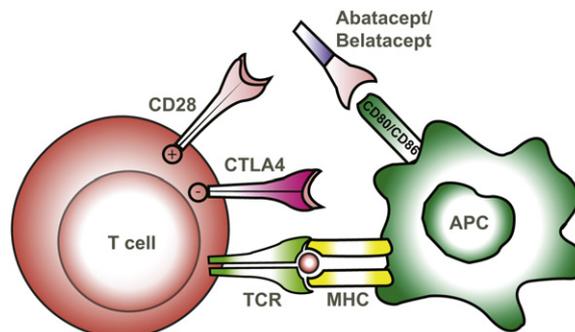


FIG 3. Abatacept and belatacept bind to CD80 and CD86 and block costimulation.

autoimmune disease. Interest has now shifted to targeting CD40, which should not be associated with thrombotic events. Preclinical studies are being conducted in autoimmune diseases and organ transplantation.

THE CD28 PATHWAY

CD28 signals promote T-cell differentiation into T_H1 phenotype cells and enhance both the production of antibodies by B cells and the proliferation of previously activated T cells.¹⁴ The 2 ligands for CD28, CD80 and CD86, have distinct but overlapping functions. CD86 is constitutively expressed and is rapidly upregulated on APCs coincident with Signal 1, whereas CD80 expression levels are very low on the resting cell; higher expression is usually induced after more prolonged T-cell stimulation.^{15,16} These differences suggest distinct roles for the 2 ligands. CD86 may be important in mediating initial T-cell activation, and CD80 may play a greater part in perpetuating the immune response. After T-cell activation, a second receptor, CTLA4, is upregulated.¹⁴ CTLA4 is structurally homologous to CD28 but has higher avidity to the CD80 and CD86 ligands and, unlike CD28, is a negative regulator of T cells. Thus, the upregulated CTLA4 successfully competes for the CD80/CD86 ligands and turns off T-cell activation. The receptor fusion protein CTLA4-Ig (consisting of the extracellular binding domain of CTLA4 linked to a

modified Fc domain of human IgG₁) has been developed to inhibit costimulation by binding to CD80/CD86.

Blockade of the binding of CD28 to CD80/CD86 has also been attempted with dual antibodies targeting both CD80 and CD86. Experimentally, these antibodies block costimulation and provide robust immunosuppression. However, clinical development of anti-CD80/anti-CD86 has been halted as a result of the increased expense of the simultaneous administration of 2 mAbs, because either ligand can trigger CD28 activation, and the vagaries of the intellectual property surrounding CD80/CD86.

A reagent that selectively targets CD28 could be a more desirable therapeutic than CTLA4-Ig because it would allow the T cell to receive inhibitory signals through CTLA4 when bound to CD80/CD86. An obvious approach is to target CD28 with mAbs. Vanhove et al¹⁷ showed that direct blockade of CD28 could in fact inhibit the mixed lymphocyte culture *in vitro*. A rat model of transplantation showed that anti-CD28 therapy induced tolerance. However, most antibodies to CD28 are agonistic or superagonistic, triggering CD28 activation in the absence of Signal 1. A superagonist anti-CD28 mAb, TGN1412, which in preclinical studies produced marked expansion of T-regulatory cells without affecting the T inflammatory component, was recently reported to have produced a *cytokine storm* and multiorgan failure in 6 volunteers.¹⁸ Another anti-CD28 antibody, FK734, a partial agonist

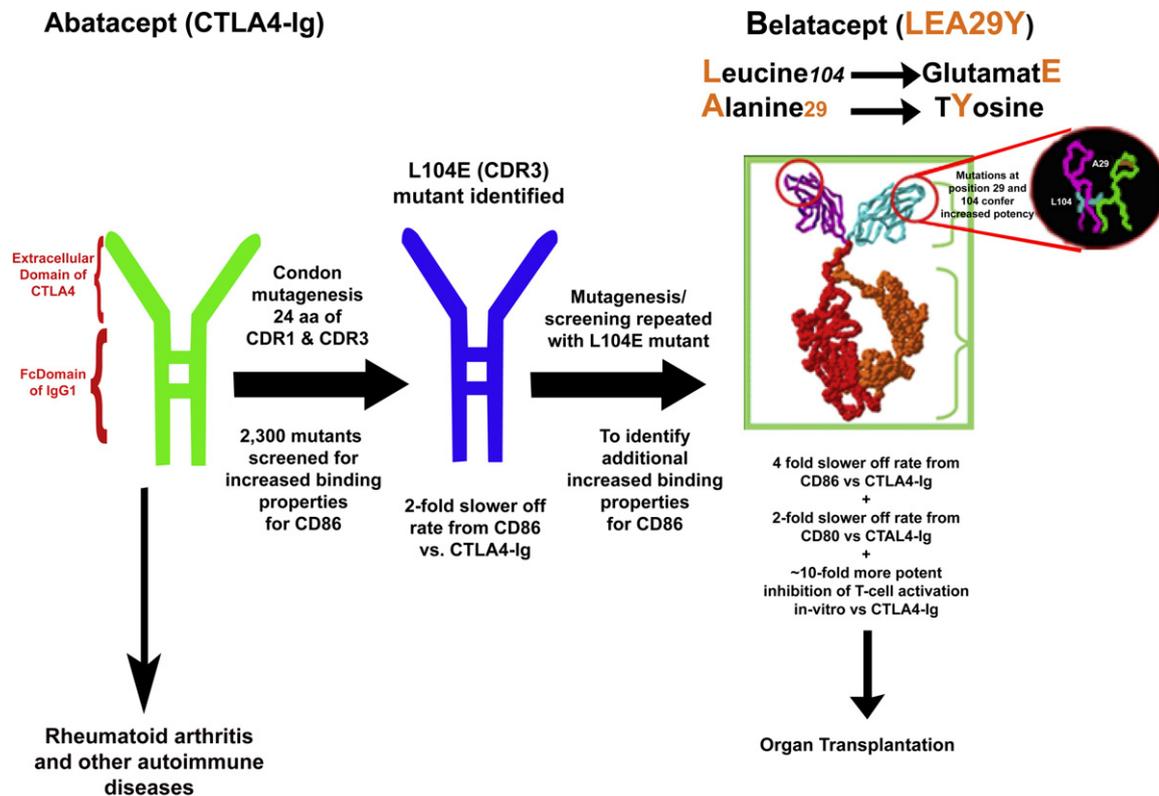


FIG 4. The development of abatacept and belatacept.

of CD28, reduced T-cell-mediated skin allograft *rejection* in rodents, but it is unlikely to be developed for clinical use.¹⁹

DEVELOPMENT OF ABATACEPT AND BELATACEPT

The dimeric fusion protein abatacept (CTLA4-Ig) was developed to block the interactions of CD28 with CD80 and CD86 (Fig 3). Abatacept binds more avidly than CD28 to CD80/CD86, as does native CTLA4. The addition of the IgG₁ domain solubilizes the CTLA4 domain, creating a soluble receptor for CD80/CD86.⁷ Numerous studies have shown that abatacept acts to inhibit immune responses both *in vitro* and *in vivo*. The *in vitro* binding of CTLA4-Ig to CD80 and CD86 downregulates T-cell proliferation and inhibits humoral immune responses.⁷⁻¹⁰

Therapeutic inhibition of the CD28 pathway by using CTLA4-Ig was first established in the transplantation setting, where graft survival was effectively prolonged and, in many cases, donor-specific tolerance was induced. Unfortunately, these effects in rodent models of transplantation could not be reproduced in the more robust models of renal transplantation studies in nonhuman primates.²⁰

The lack of efficacy of CTLA4-Ig in organ transplantation in nonhuman primates was likely related to the lower avidity of CTLA4-Ig to CD86 compared with CD80. For this reason, a rational design concept was used to improve the binding characteristics of CTLA4-Ig to CD86 specifically, because of the presumed importance of CD86 in the initiation of the alloimmune reaction.²⁰ A mutagenesis and screening strategy was used to develop belatacept (Fig 4). Codon-based mutagenesis of CTLA4-Ig was performed on 24 individual amino acids within the complementary determining region (CDR) and CDR3 analogous loops (ie, not antibody-derived CDRs, but the analogous regions on the CTLA4 portion of abatacept), and the region C-terminal to the

CDR3-like loop, because these regions were previously identified as critical for high-avidity receptor binding. A total of 2300 mutants were screened by using a binding assay, and a variant with a slower off rate was identified. This variant, which had a leucine to glutamate switch at position 104, was then used in a second round of mutagenesis and screening. The second round yielded belatacept with a second mutation (alanine to tyrosine) at position 29.

This process resulted in belatacept, a second-generation CTLA4-Ig, which demonstrated superior binding to CD80 and CD86 compared with CTLA4-Ig and provided the more potent immunosuppressive properties required for transplantation (Fig 4). Abatacept, the parent molecule, was shown to be effective in the treatment of autoimmune diseases and has been approved by the FDA for the treatment of moderate to severe rheumatoid arthritis (RA).^{4,5,21,22}

CURRENT TREATMENTS FOR RA

Current treatment options for RA are multifocal and include nonsteroidal anti-inflammatory drugs, corticosteroids, and the so-called *disease-modifying anti-inflammatory drugs* (DMARDs), such as hydroxychloroquine, sulfasalazine, and methotrexate. Methotrexate is the mainstay of RA treatment. Recently, protein therapeutics, or biologic DMARDs, which specifically target key inflammatory cytokines, have been shown to be effective in treating RA. Of these, 3 inhibit *TNF-α* (adalimumab, infliximab, and etanercept), and the fourth inhibits *IL-1* (anakinra).

However, 20% to 40% of patients who receive anti-TNF therapies have no clinical response as measured by the American College of Rheumatology criteria, and some patients may lose their response over time.²³ In addition, serious side effects have emerged in some patients treated with these biologics.

TABLE I. Efficacy of abatacept in patients with RA with an inadequate response to methotrexate and/or TNF- α inhibitors

Outcome	Clinical efficacy at 1 year in patients with an inadequate response to methotrexate (AIM study)		Clinical efficacy at 6 months in patients with an inadequate response anti-TNF treatment (ATTAIN study)	
	Abatacept + methotrexate N = 424	Placebo + methotrexate N = 214	Abatacept + DMARDs N = 256	Placebo + DMARDs N = 133
20% Improvement in ACR criteria (% ACR 20)	73	40	50	20
50% Improvement in ACR criteria (% ACR 50)	48	18	20	4
70% Improvement in ACR criteria (% ACR 70)	29	6	10	2
Remission by DAS28 criteria (% DAS28 <2.6)	24	2	10.0	0.8
Low disease activity by DAS28 criteria (% DAS28 \leq 3.2)	42.5	10	17.1	3.1
Clinically meaningful improvement in physical function (% HAQ improvement \geq 0.3 from baseline)	64	39	47.3	23.3
Mean change from baseline in the physical component summary score of the SF-36	9.1	5.0	6.59	1.12
Mean change from baseline in the mental component summary score of the SF-36	6.9	4.7	5.15	2.11
Radiographic scores (mean change from baseline)				
Erosions	0.63	1.14		
Joint space narrowing	0.58	1.18		
Total score	1.21	2.32		
Discontinuation due to lack of efficacy (%)	3	9	20	5

ACR, American College of Rheumatology; AIM, Abatacept in Inadequate responders to Methotrexate; ATTAIN, Abatacept Trial in Treatment of Anti-TNF Inadequate responders; DAS28, Disease Activity Score 28; HAQ, Health Assessment Questionnaire; SF-36, Short Form 36.

COSTIMULATION MODULATION IN AUTOIMMUNE DISEASES

The efficacy of abatacept in treating human autoimmune diseases was first demonstrated in patients with *psoriasis vulgaris*, in whom a beneficial effect was demonstrated on psoriatic lesions.²¹ Phase II and III trials of abatacept plus methotrexate in patients with RA with an inadequate response to methotrexate demonstrated statistically significant and clinically meaningful improvements in the signs and symptoms of RA compared with methotrexate alone^{24,25} (Table I). Statistically significant and clinically meaningful improvements were also seen in physical function and both the mental and physical aspects of Health Related Quality of Life. These benefits were sustained and, in some cases, augmented in patients who continued through 5 years of open-label treatment during a long-term extension of the phase II trial.²⁶ Abatacept also inhibits the progression of structural damage at 1 year compared with methotrexate.²⁷

As with the anti-TNF agents, not all patients responded to abatacept, although the proportion of responders was similar to that seen in studies of anti-TNFs. However, abatacept is the first agent to be studied and approved for the treatment of patients with an inadequate response to treatment with TNF- α antagonists, on the basis of the data from the phase III Abatacept Trial in Treatment of Anti-TNF Inadequate responders study.⁵ In these patients, abatacept demonstrated statistically significant and clinically meaningful improvements in the reduction of signs and symptoms and improvements in physical function and Health Related Quality of Life (Table I). When data from 5 clinical trials of abatacept in 4000 patients with RA representing more than 8000 patient-years were integrated, abatacept was found to be well tolerated, with a consistent safety profile.²⁸ The incidence rates of total malignancies and individual malignancies in the abatacept clinical program were within the ranges found in patients with RA, analyzed by using several well

established databases representing large RA cohorts from different sources.

Abatacept is approved for use in patients with an inadequate response to anti-TNF agents and also for patients with an inadequate response to 1 or more DMARDs such as methotrexate. Thus, it is not exclusively approved or used after TNF agents. It is typically used with DMARDs, as are the TNF agents.

The simultaneous use of abatacept and anti-TNF therapy is not currently advised because the few patients treated with this combination had a higher incidence of serious infections without an apparent improvement in the outcome.

Antibodies to the immunoglobulin portion or CTLA4-binding portions were reported in 1.3% of patients and showed low-level reactivity.

SOLID-ORGAN TRANSPLANTATION Overview

Recipients of solid-organ transplants generally require lifelong immunosuppression to maintain a state of low immunoresponsiveness or nonimmunoresponsiveness to the allograft. Unfortunately, advances in the regimens and therapies used to prevent rejection have not been matched by similar improvements in long-term patient or graft survival.²⁹

In the 1990s, several immunosuppression agents, small molecules and biologics, were successfully introduced in the clinic. These new immunosuppression regimens produced a dramatic decrease in antirejection rates from the 40% range to the 10% to 15% range.²⁹ Because rejection is a strong risk factor for graft loss, the decrease in the incidence of acute rejection was anticipated to lead to a significant improvement in long-term graft survival. Yet to date, outcome data from the Scientific Registry of Transplant Recipients have not demonstrated an increase in long-term graft survival in the recipients of primary kidney transplants.²⁹

Paradoxically, the long-term survival of both the allografts and the recipients are affected by nonimmune toxicities caused, in part, by the immunosuppressive therapies used to prevent graft rejection. Current immunosuppressive regimens are associated with increased risks of diabetes mellitus, cardiovascular disease, and malignancies. Perhaps most critically, calcineurin inhibitors (CNIs), currently the cornerstone therapy for chronic maintenance of immunosuppression, are almost invariably associated with nephrotoxicity, resulting in a high incidence of renal failure and intensifying the already considerable supply/demand imbalance.^{30,31}

Current therapeutics in transplantation

The immune response to transplantation of an allograft is primarily mediated through T-cell–dependent mechanisms, including cytokine expression, T-cell proliferation, and clonal expansion, as well as some forms of antibody-mediated rejection that require T-cell interaction for initiation.

The cascade of events that lead to organ rejection presents a number of targets for inhibiting T cells, T-cell activation, and the subsequent effects of activated T cells. Current immunosuppressive strategies inhibit T-cell effects by depleting or modulating T cells, inhibiting T-cell signaling pathways, blocking T-cell proliferation, or interrupting the trafficking of T cells into allografts/injured tissue.

Regimens generally use more potent immunosuppression in the early posttransplant or induction phase, when immune responses to the allograft are at their highest. Induction agents include protein therapeutics (either polyclonal antibodies or murine, chimeric, or humanized mAbs), which act mainly by depleting lymphocytes or suppressing proliferation and blunting the effects of T-cell activation. Protein therapeutics target specific cell surface glycoproteins or membrane-bound receptors and fall into 2 broad categories: depleting and nondepleting (ie, modulating) agents.

Depleting biologics such as the antithymocyte globulin and OKT3 are used to reduce early rejection by depleting T cells at the time of antigen presentation and thus preventing activation of alloreactive T-cell clones. They can be associated with cytokine release syndrome as well as the immunologic consequences of prolonged T-cell depletion, such as infectious complications and *posttransplant lymphoproliferative disease (PTLD)*.

Daclizumab and basiliximab are nondepleting mAbs. These agents specifically target the α -chain of the IL-2 receptor (IL-2R), inhibiting the signal for proliferation resulting from initial T-cell activation. Unlike the T-cell–depleting agents, these compounds are associated with a low incidence of toxicities and side effects.³² However, despite their specificity, the anti-IL-2R mAbs have limited efficacy because of the redundancy of the cytokine receptor system and are generally inappropriate for long-term use.³³ The depleting agents act too broadly, compromising safety; the anti-IL-2R antibodies act too narrowly (targeting only 1 aspect of T-cell activation) effectively to block rejection when used without calcineurin inhibitors.

After the induction phase, transplant patients transition to what is known as the maintenance phase of immunosuppressive treatment.³⁴ Effective maintenance immunosuppression is the key to preventing rejection throughout the life of the graft. Maintenance immunosuppressive strategies typically include the so-called *cornerstone therapies*, CNIs, along with antiproliferative agents (mycophenolate mofetil [MMF]/enteric-coated mycophenolate acid or sirolimus) and steroids.

The CNI regimens necessitate frequent therapeutic drug monitoring and dosage adjustment to remain within the narrow therapeutic windows and are associated with multiple toxicities.³⁴

These limitations have motivated a search for novel agents/regimens with greater selectivity and improved toxicity profiles. Recently, as with autoimmune diseases, the focus of research has turned toward costimulation blockade.

Thus, the promise of costimulation blockade is to provide selective but durable immunosuppression without the nephrotoxicity and metabolic toxicity of current agents that can ultimately translate into improved long-term patient and graft survival.

Costimulation blockade in transplantation

Preclinical studies demonstrated CTLA4-Ig–mediated inhibition of T-cell–dependent antibody responses and prolongation of transplanted organ survival.³⁵ However, CTLA4-Ig was found to be inadequate to maintain a hyporesponsive state to an allograft in some models.²⁰ It has been demonstrated that CD80 and CD86 may differentially control the immune response because of the distinct properties of each molecule.³⁶ The more rapid dissociation of CTLA4-Ig from CD86 than from CD80 may have resulted in less effective inhibition of CD86-dependent responses than of CD80-dependent responses.³⁷ In addition, anti-CD86 antibodies, but not anti-CD80 antibodies, prevented the rejection of allogeneic islet transplants, although the combination of both antibodies was best able to prolong allograft survival.³⁸ Therefore, it was hypothesized that a compound that bound to CD86 with higher avidity than CTLA4-Ig would provide the inhibition of T-cell costimulation necessary to prevent allograft rejection. This led to the development of belatacept (LEA29Y), a modified version of abatacept, which was rationally designed to provide the CD86-binding properties required for immunosuppression in transplantation (Fig 4).²⁰

In nonhuman primate renal transplant studies, belatacept demonstrated better efficacy in preventing acute rejection compared with abatacept. When combined with drugs typically used in human transplant immunosuppressive regimens—basiliximab (an anti IL-2R antibody), steroids, and MMF (an antiproliferative)—renal allograft function was prolonged.²⁰ Belatacept also inhibited the formation of antidonor antibodies, thought to contribute to the development of chronic rejection and a major barrier to retransplantation.

The findings in nonhuman primate renal transplantation were used to design a phase II multicenter clinical study comparing the safety and efficacy of belatacept versus cyclosporine.⁶ The design of this trial was novel, because previous biologic agents have been used for a short term as induction agents perioperatively, whereas belatacept was to be administered chronically in maintenance therapy.

De novo renal transplant recipients received either a more or less intensive belatacept dosing regimen or cyclosporine. All patients received basiliximab induction, MMF, and corticosteroids. At 6 months, there was no significant difference in the incidence of clinically suspected, biopsy-proven acute rejection among the 3 treatment groups, despite the complete avoidance of CNIs in the belatacept arms.

Importantly, renal function at 12 months, shown to be the most important predictor of the long-term survival of renal allografts,³⁹ was significantly better preserved in patients receiving belatacept than in patients receiving cyclosporine, possibly because of the avoidance of CNIs. Furthermore, belatacept-treated patients demonstrated significantly lower rates of tubular atrophy and interstitial fibrosis (also known as *chronic allograft nephropathy*) on histologic examination. Chronic allograft nephropathy is almost

universally present 10 years posttransplant in patients treated with CNIs and is an important factor in allograft loss.²⁹ Therefore, the renal function benefits seen with belatacept, if borne out in the long term and in the current phase III studies with higher patient numbers, are likely to prove extremely significant.

Three patients treated with belatacept PTLD. All were in the more intense treatment arm 1. In 2 patients, the PTLD was associated with primary EBV infection. Although this is a serious complication, it is not yet known whether this is an important safety signal or a random cluster of PTLD cases. The complication will require confirmation from the phase III studies. Some reassurance was recently provided by the long-term follow-up of the phase II patients who consented to be in the long-term extension trial.⁴⁰ A total of 102 patients on belatacept were followed with the same regimen for a median of 48 months. No additional cases of PTLD were reported. At 1 year, antibodies to belatacept were not detected.

The ability to use an immunospecific maintenance immunosuppression regimen that improves long-term outcomes may herald a new era in patient care after transplantation. Phase III clinical trials of belatacept are ongoing in renal transplantation, both in primary renal allograft recipients and in recipients using organs from extended criteria donors. The latter recipients have a particularly high unmet need because of the increased susceptibility of these organs to the nephrotoxic effects of CNIs. The results of these phase III studies and trials in additional organs are eagerly anticipated in 2008 and 2009.

Future trials with costimulation blockade

Beyond RA, costimulation blockade is likely to be tested or used off-label in other autoimmune diseases including SLE, multiple sclerosis, and potentially allergic conditions such as asthma.⁴¹

An important challenge with belatacept therapy in organ transplantation is the determination of the optimum concomitant immunosuppression to facilitate operational tolerance. It is likely that in addition to belatacept, other costimulation or adhesion molecules may need to be targeted for optimal results.²¹ Experimentally costimulation blockade and anti-LFA1 therapy resulted in prolonged graft survival. However, these interesting combinations of biologics will likely be tested clinically after FDA approval of belatacept.

REFERENCES

- Bretscher PA. A two-step, two-signal model for the primary activation of precursor helper T cells. *Proc Natl Acad Sci U S A* 1999;96:185-90.
- Sayegh MH, Turka LA. The role of T-cell costimulatory activation pathways in transplant rejection. *N Engl J Med* 1998;338:1813-21.
- Linsley PS, Ledbetter JA. The role of the CD28 receptor during T cell responses to antigen. *Annu Rev Immunol* 1993;11:191-212.
- Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4-Ig. *N Engl J Med* 2003;349:1907-15.
- Genovese M, Becker J-C, Schiff M, Luggen M, Sherrer Y, Kremer J, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med* 2005;353:1114-23.
- Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blanche G, et al. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005;353:770-81.
- Judge TA, Tang A, Spain LM, Deans-Gratiot J, Sayegh MH, Turka LA. The in vivo mechanism of action of CTLA4-Ig. *J Immunol* 1996;156:2294-9.
- Webb LM, Walmsley MJ, Feldmann M. Prevention and amelioration of collagen-induced arthritis by blockade of the CD28 costimulatory pathway: requirement for both B7-1 and B7-2. *Eur J Immunol* 1996;26:2320-8.
- Lenschow DJ, Zeng Y, Thistlethwaite JR, Montag A, Brady W, Gibson MG, et al. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4 Ig. *Science* 1992;257:789-92.
- Pearson TC, Alexander DZ, Winn KJ, Linsley PS, Lowry RP, Larsen CP. Transplantation tolerance induced by CTLA4-Ig. *Transplantation* 1994;57:1701-6.
- Vincenti F. Chronic induction: what's new in the pipeline. *Contrib Nephrol* 2005;146:22-9.
- Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* 1998;280:243-8.
- Yamada A, Salama AD, Sayegh MH. The role of novel T cell costimulatory pathways in autoimmunity and transplantation. *J Am Soc Nephrol* 2002;13:559-75.
- Alegre ML, Frauwrath KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. *Nat Rev Immunol* 2001;1:220-8.
- McAdam AJ, Schweitzer AN, Sharpe AH. The role of B7 costimulation in activation and differentiation of CD4+ and CD8+ T cells. *Immunol Rev* 1998;165:231-47.
- Kim KS, Denton MD, Chandraker A, Knoflach A, Milord R, Waaga AM, et al. CD28-B7-mediated T cell costimulation in chronic cardiac allograft rejection: differential role of B7-1 in initiation versus progression of graft arteriosclerosis. *Am J Pathol* 2001;158:977-86.
- Vanhove B, Laflamme G, Coulon F, Mouglin M, Vusio P, Haspot F, et al. Selective blockade of CD28 and not CTLA-4 with a single-chain Fv-alpha1-antitrypsin fusion antibody. *Blood* 2003;102:564-70.
- Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, et al. Cytokine storm in a phase I trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018-28.
- Shiao SL, McNiff JM, Masunaga T, Tamura K, Kubo K, Pober JS. Immunomodulatory properties of FK734, a humanized anti-CD28 monoclonal antibody with agonistic and antagonistic activities. *Transplantation* 2007;83:304-13.
- Larsen CP, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobertm E, et al. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant* 2005;5:443-53.
- Abrams JR, Lebwohl MG, Guzzo CA, Jegasothy BV, Goldfarb MT, Goffe BS, et al. CTLA4-Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J Clin Invest* 1999;103:1243-52.
- Finck BK, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA4-Ig. *Science* 1994;265:1225-7.
- Sidiropoulos P, Bertsias G, Kritikos HD, Kouroumali H, Voudouris K, Boumpas DT. Infliximab treatment for rheumatoid arthritis, with dose titration based on the Disease Activity Score: dose adjustments are common but not always sufficient to assure sustained benefit. *Ann Rheum Dis* 2004;63:144-8.
- Kremer JM, Dougados M, Emery P, Sibilia J, Shergy W, et al. Treatment of rheumatoid arthritis with the selective costimulation modulator abatacept: twelve-month results of a phase IIb, double-blind, randomized, placebo-controlled trial. *Arthritis Rheum* 2005;52:2263-71.
- Kremer JM, Genant HK, Moreland LW, Russell AS, Emery P, Abud-Mendoza C, et al. Effects of abatacept in patients with methotrexate-resistant active rheumatoid arthritis: a randomized trial. *Ann Intern Med* 2006;144:865-76.
- Westhovens R, Emery P, Aranda R. Abatacept provides sustained clinical benefit through 3 years in rheumatoid arthritis patients with inadequate responses to methotrexate [abstract SAT0203]. *Ann Rheum Dis* 2006;65(suppl II):512.
- Genant HK, Peterfy C, Wu C. An ACR 20 response is not required for inhibition of structural damage progression by abatacept: results from the AIM Trial [abstract 1991]. *Arthritis Rheum* 2005;52(suppl 9):S350.
- Weinblatt ME, Combe B, Covucci A, Aranda R, Becker JC, Keystone E, et al. Safety of the selective costimulation modulator abatacept in rheumatoid arthritis patients receiving background biologic and nonbiologic disease-modifying antirheumatic drugs: a one-year randomized, placebo-controlled study. *Arthritis Res* 2006;54:2807-16.
- Meier-Kriesche H-U, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Amer J Transplantation* 2004;4:378-83.
- Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, et al. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003;349:931-40.
- Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation* 2004;78:557-65.
- Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. *N Engl J Med* 1998;338:161-5.
- Vincenti F, Ramos E, Brattstrom C, Cho S, Ekberg H, Grinyo J. Multicenter trial exploring calcineurin inhibitors avoidance in renal transplantation. *Transplantation* 2001;71:1282-7.
- Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004;351:2715-29.
- Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, et al. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci U S A* 1997;94:8789-94.

36. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 1996;14:233-58.
37. Linsley PS, Greene JK, Brady W, Bajorath J, Ledbetter JA, Peach R, et al. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity* 1994;1:793-801.
38. Lenschow DJ, Zeng Y, Hathcock KS, Zuckerman LA, Freeman G, Thistlethwaite JR, et al. Inhibition of transplant rejection following treatment with anti-B7-2 and anti-B7-1 antibodies. *Transplantation* 1995;60:1171-8.
39. Hariharan S, McBride MA, Cherikh WS, Tolleris CB, Bresnahan BA, Johnson CP. Post-transplant renal function in the first year predicts long-term kidney transplant survival. *Kidney Int* 2002;62:311-8.
40. Vincenti F, Larsen C, Muhlbacher F, Mark D, Wang Y, Listman J, et al. 2007. Interim report of phase 2 long-term safety of belatacept [abstract 0253]. Presented at European Society of Organ Transplantation; September 29-October 3, 2007; Prague, Czech Republic.
41. Krinzman SJ, De Sanctis GT, Cernadas M, Mark D, Wang Y, Listman J, et al. Inhibition of T cell costimulation abrogates airway hyperresponsiveness in a murine model. *J Clin Invest* 1996;98:2693-9.