After a very long and windy road, in December of 2005 the FDA approved CTLA4Ig for the treatment of rheumatoid arthritis. Ocrenza is the first-in-class antagonist of CD28 costimulation. In this perspective, we discuss the science that led to CTLA4Ig development and the clinical challenges in bringing the drug from the bench to the bedside.

Introduction

T cell costimulation is among the most important concepts to emerge in basic immunology in the past 30 years. It proposes that an antigen-presenting cell (APC) must provide two independent signals for full T cell activation. Signal one is T cell receptor (TCR) stimulation, provided by antigen (Ag) bound to a major histocompatibility complex molecule (Ag-MHC). Signal two is provided by a ligand for a T cell-expressed costimulatory receptor (Lafferty et al., 1983; Lafferty and Woolnough, 1977). Together, both signals lead to optimal cytokine production, proliferation, effector function, and survival (June et al., 1994). In contrast, costimulation alone in the absence of cognate antigen has no effect on the T cell (June et al., 1994), while provision of signal one (TCR) without signal two (costimulation) leads to active induction of T cell unresponsiveness or anergy (Jenkins and Schwartz, 1987). This paradigm has two important implications. First, as only “professional” APCs can provide costimulation, interactions of T cells with self-antigen-bearing stromal tissues do not generally induce activation, providing a mechanism for self tolerance even in the presence of an autoreactive T cell repertoire. Second, the concept of costimulation identified new important targets for drug development because tolerance might be achieved by blocking costimulation, even if the relevant antigens were numerous (transplantation) or not known (many autoimmune diseases). Thus, targeting costimulatory receptors became the mantra of the immune tolerance community.

Over the past two decades, a number of molecules have been implicated as costimulatory receptors or ligands for naive T cells (Greenwald et al., 2005; Quezada et al., 2004). Although interactions such as CD154 with its ligand, CD40, have profound effects on immune responses, the monovalent homodimer CD28, which interacts with CD80 (B7-1) and CD86 (B7-2), remains the most prominent costimulatory receptor for naive T cells by providing both a qualitatively distinct second signal and amplifying the transcriptional effects of TCR triggering (Lenschow et al., 1996; Salomon and Bluestone, 2001).

Here we summarize the studies of the CD28-costimulatory antagonist CTLA4Ig, the first of its class brought to market, beginning with its topsy-turvy roller coaster ride through preclinical and clinical development and culminating with drug approval. We also highlight the critical basic and clinical observations that have synergized to advance the field and propose future avenues of research that may further exploit this new class of immunoregulatory drugs.

CTLA4Ig as a Potent Inhibitor of CD28-Mediated T Cell Costimulation

B7-1 (CD80) was the first ligand for CD28 identified, leading to the use of anti-B7-1 antibodies to block costimulation (Gimmi et al., 1991). However, in several systems the antibody was ineffective, which we now know to be due to the presence of a second CD28 ligand, B7-2 (CD86) (Freeman et al., 1993; Lenschow et al., 1996; Salomon and Bluestone, 2001). To circumvent this problem, Linsley and co-workers developed a soluble CD28 fusion protein (Peach et al., 1994), CD28lg, with the expectation that it would block CD28 interactions with both CD80 and CD86. However, this molecule was ineffective, mainly because of the low affinity of CD28 for its ligands. Recent structural analysis has confirmed that the weak monomeric interactions that exist between CD28 and its ligands result in poor binding in the absence of a high degree of multivalency such as that present in the immunologic synapse during T cell-APC interactions (Jansson et al., 2005; van der Merwe and Davis, 2003).

At about the same time, Tullia Lindsten and Craig Thompson showed that a newly discovered protein, CTLA-4 (Brunet et al., 1987), shared many attributes of CD28, including a six amino acid sequence (MYPPPY) that was critical for binding to CD80 and CD86 (Lindsten et al., 1993). Because CTLA-4 had a higher affinity for CD80 and CD86 than CD28, the soluble fusion protein CTLA4Ig became the therapy of choice for blocking CD28-B7 interactions (Figure 1). CTLA4Ig binds effectively to both CD80 and CD86, although a number of biophysical and structural studies suggest that CD86 preferentially engages CD28 at the synapse, in contrast to CD80, which ligates CTLA-4 more effectively than CD28 (Linsley et al., 1991). In fact, CD80 completely dominates interactions with CTLA-4, forming linear arrays of receptor-ligand pairs.

Despite its homology to CD28, we and others observed that CTLA-4 is in fact a negative regulator of CD28-mediated T cell costimulation (Krummel and Allison, 1995; Walunas et al., 1994). Importantly, resting T cells and APCs primarily express CD28 and CD86, respectively, while activation induces CTLA-4 and CD86 (Jansson et al., 2005; van der Merwe and Davis, 2003).
Thus, differences in binding avidity of CD28 and CTLA-4 for CD80 and CD86 are critical to understanding the temporal nature of the immune response, suggesting a model in which positive signals delivered via CD28:CD86 dominate initially, while negative signals resulting from CTLA-4:CD80 ligation serve a counterregulatory function to terminate T cell responses. Thus, CTLA4Ig, through its binding to CD80 and CD86, inhibits both costimulation through CD28 as well as negative signaling via CTLA-4, potentially leading to diverse outcomes depending on the stage of T cell activation.

The development of this effective CD28 antagonist prompted studies testing its efficacy in vivo. In several different transplant settings, we and others showed that short-term CTLA4Ig therapy led to long-term tissue and organ graft survival, suppression of alloantibody responses, and, most importantly, induction of tolerance (Larsen et al., 1996; Lenschow et al., 1992; Lin et al., 1993). Similar observations in animal models of autoimmunity suggested that CD28 blockade not only functioned at the initiation of an immune response but could also ameliorate ongoing immunity (Salomon and Bluestone, 2001). Most importantly, in a substantial percentage of these studies, CTLA4Ig therapy could be stopped after a short time, and its effects remained long lived.

Over the last 15 years, the mechanistic basis for CTLA4Ig function in vivo has become increasingly clear. First and foremost, CTLA4Ig blocks the engagement of CD28 with its ligands CD80 and CD86, inhibiting the early phases of T cell activation, including progression into cell cycle, effector differentiation, and cell survival (Salomon and Bluestone, 2001). However, the original work showing that blockade of T cell costimulation in vitro results in T cell anergy has not been borne out in vivo. Individual T cells isolated from treated animals are able to function ex vivo (Szot et al., 2000). Rather, we showed that a major effect of costimulation blockade in vivo is to promote passive (growth factor withdrawal) cell death and limit the clonal expansion of antigen-reactive T cells (Lakkis et al., 1997; Li et al., 1999; Wells et al., 1999) and that tolerance induction is critically dependent on this proapoptotic activity.

In addition to these major functions, other in vivo effects of CTLA4Ig have been described. CTLA4Ig functions in allogeneic islet transplant tolerance by triggering APC production of indoleamine 2, 3-dioxygenase (IDO), an intracellular enzyme that breaks down tryptophan and suppresses T cell activation (Grohmann et al., 2002). These data provide an interesting link with fetal-maternal tolerance, which is also IDO dependent (Mellor and Munn, 2004) and suggests multiple ways in which immune regulation following CTLA4Ig treatment may occur. Moreover, under certain circumstances, CTLA4Ig can augment immunity. This may occur for two reasons. First, CTLA4Ig treatment can block ligation of the negative regulator, CTLA-4, resulting in enhanced T cell activation (Salomon and Bluestone, 2001). More importantly, CTLA4Ig treatment has direct effects on regulatory T cells (Tregs), which are critically important in the control of autoimmunity and many settings of transplant tolerance (Salomon et al., 2000). The development and peripheral survival of Tregs is CD28 dependent; thus, CTLA4Ig treatment results in a precipitous reduction of Tregs and in some cases exacerbation of autoimmunity (Tang et al., 2003). It is unclear whether these potential adverse effects of CTLA4Ig therapy will be a critical issue in the human setting. It is likely to depend on location, timing, and the relevant importance of CTLA-4-expressing activated T cells and Tregs in controlling immunity in the different diseases. In this

![Figure 1. Multiple Effects of CTLA4Ig on Immune Function](#)

CTLA4Ig functions to block the interaction of CD28-B7 during activation of naive and activated helper or cytotoxic T cells (2). The CD28 blockade results in increased cell death, anergy induction, and blockade of cell differentiation. CTLA4Ig also has another potentially immunosuppressive effect by interacting with B7 on dendritic cells inducing IDO, which indirectly blocks naive cell activation (1). Finally, CTLA4Ig can potentially have an immune augmenting effect by blocking the interaction of CTLA-4 with the same B7 ligands on effector cell function (3) or blockade of CD28-B7 interactions on regulatory T cell function (4), which results in enhanced pathogenic T cell activity.
context, it is interesting to note that anti-CD86 monoclonal antibody therapy is quite efficacious in several models due to its dramatic effects on CD28 costimulation with limited affects on CTLA-4 engagement or Treg survival (Bour-Jordan et al., 2005; Haanstra et al., 2003; Kirk et al., 2001).

**Moving the Drug to the Clinic**

The studies of CTLA4Ig in rodent models cited above were extremely encouraging; however, the pipeline is littered with drugs that ameliorate mouse diseases but later fall victim to the rigors of clinical testing. What separates CTLA4Ig from other promising efforts to modulate T cell activation is that this agent, known by the name abatacept (Orenica, Bristol-Myers Squibb, New York, New York), is now an approved drug for the treatment of rheumatoid arthritis (http://www.fda.gov/cder/foi/appletter/2005/125118rev2.pdf).

Reaching this point was not easy—the development path had many twists and turns as Bristol-Myers Squibb was challenged legally, clinically, and in drug manufacturing. Initial studies focused in the transplant arena using nonhuman primates. Compared to the results from murine studies, CTLA4Ig was far less efficacious in this setting (Kirk et al., 1997; Levisetti et al., 1997). The drug was not able to block graft rejection alone unless used continuously. The drug appeared to affect alloantibody responses better than T cell-mediated alloreactive rejection. This finding was consistent with mouse studies showing a profound effect of CTLA4Ig on Th2 immunity; however, with few exceptions, treatment with CTLA4Ig failed to induce tolerance in the nonhuman primate models or to produce long-term graft survival. Meanwhile, another company, Biogen, Inc. (now Biogen Idec, Cambridge, Massachusetts) was racing forward to develop a monoclonal antibody to CD40L (CD154), a potent costimulatory blocker in preclinical animal models. In studies in nonhuman primates using the combination of CTLA4Ig and anti-CD40L, blockade of the CD40L pathway seemed to provide greater suppression of immune responses (Kirk et al., 1997, 1999).

It appeared initially that Bristol-Myers Squibb would not continue with the development of CTLA4Ig. However, several events changed the landscape. First, in phase II clinical trials, anti-CD40L treatment was associated with unanticipated side effects (Sidropoulos and Boumpas, 2004). A few patients treated with the drug experienced thromboembolic events most likely due to reactivity of the monoclonal antibody with CD40L expressed on the surface of activated platelets. Second, Bristol-Myers Squibb received encouraging news from a phase I open-label dose-escalation study of CTLA4Ig therapy in patients with psoriasis vulgaris (Abrams et al., 1999), a T cell-dependent skin disorder characterized by abnormal keratinocyte proliferation and differentiation, and an inflammatory infiltrate of T cells and APCs at the dermal-epidermal junction. Nearly half of the patients in this study achieved 50% or more improvement in clinical disease activity, with greater response rates in the cohorts receiving the highest doses. Pre- and post-treatment skin biopsies showed that this treatment effect was accompanied by normalization of keratinocyte proliferation and maturation, a reduction in the numbers of infiltrating T cells, and diminished expression of CD40 and HLA-DR antigens by keratinocytes and of CD80, CD86, and MHC class II antigens by dendritic cells (Abrams et al., 2000).

Importantly as well, CTLA4Ig therapy was proving to have an acceptable toxicity profile. It did not appear to decrease serum Ig concentrations (Abrams et al., 2000). Flow cytometry for multiple B and T cell markers revealed no major alterations in subsets of peripheral blood lymphocytes. Because animal studies had shown that treatment with CTLA4Ig abrogated humoral immune responses to T cell-dependent antigens, the patients with psoriasis in the phase I trial were immunized with two neoantigens—KLH-Immune Activator and bacteriophage φX174. Suppression of antibody titers was observed for at least some of the patients in each dosing cohort after primary and secondary immunization with φX174, as well as secondary immunization with KLH. However, by the fourth immunization, most patients developed an immune response to bacteriophage φX174 and KLH equivalent to that of untreated individuals, indicating that CTLA4Ig therapy did not induce permanent tolerance to these antigens.

There was also progress on the transplant front. A number of groups undertook efforts to develop higher-affinity forms of CTLA4Ig that could bind both ligands effectively. Peach and colleagues observed that two amino acid mutations in the extracellular domain created a molecule, termed LEA29Y, that bound CD80 2-fold better than CTLA4Ig but, more importantly, bound CD86 4-fold better. Some have suggested that the mutant form has ten times the binding avidity for CD80 and CD86 compared with CTLA4Ig. LEA29Y showed significantly greater efficacy in the transplant setting in nonhuman primates (Larsen et al., 2005), which improved yet further when drug treatment was combined with other immune modulators (Larsen et al., 2005). Most importantly, there were relatively few serious adverse effects in these studies.

CTLA4Ig (abatacept) and LEA29Y (belatacept) have been investigated more recently in human clinical trials to prevent transplant rejection and treat rheumatoid arthritis. The work in rheumatoid arthritis led to the approval of abatacept by the US Food and Drug Administration on December 30, 2005, for this indication. The pathway toward approval of abatacept had accelerated rapidly after a phase II, randomized, double-blind, placebo-controlled, dose-finding study showed that both abatacept and belatacept therapy had potential clinical efficacy in patients with refractory rheumatoid arthritis (Moreland et al., 2002). Several large, multicenter, randomized, controlled trials were subsequently performed substantiating the clinical efficacy and safety of abatacept for the treatment of rheumatoid arthritis. Response rates were on par with those found using agents blocking tumor necrosis factor-α (TNF) (e.g., etanercept, infliximab, adalimumab), which arguably represent the most important advance in the treatment of rheumatoid arthritis over the past decade. Moreover, abatacept therapy was shown to significantly inhibit radiographic progression of disease, a surrogate measure of joint damage. The safety data from these trials continue to reveal a favorable profile, although patients receiving a combination of abatacept and another biologic (e.g., TNF blocker) had a higher incidence of serious bacterial
infections compared with those receiving nonbiologic agents or only a single biologic agent. These results are intriguing because they represent the first clinical success of a biologic therapy for rheumatoid arthritis aimed specifically at inhibiting (or depleting) T cells. Prior T cell-depleting therapies such as anti-CD4, anti-CD5, and anti-CD52 produced disappointing results in phase I and II trials, suggesting that wholesale depletion of T cells or major T cell subsets is not be effective for the treatment of rheumatoid arthritis. While cyclosporine, an inhibitor of T cell activation, has been shown to be effective for the treatment of rheumatoid arthritis, it may affect other cell types and pathogenic pathways. Whether or not other therapies for rheumatoid arthritis, such as methotrexate, ameliorate disease by inhibiting T cells is uncertain. The clinical efficacy of abatacept therapy in this setting may therefore provide new clues to the mechanisms of disease. Unknown still is how CTLA4Ig therapy reduces joint inflammation and damage in RA. Mechanisms may include blockade of CD28-mediated T cell activation, induction of IDO, or even depletion of CD80- and CD86-expressing dendritic cells via antibody- or complement-dependent cytolysis triggered by the Fc portion of the molecule. Unfortunately, clinical trials with an Fc receptor nonbinding form of CTLA4Ig (Repligen Corporation) have been halted due to intellectual property issues. Nevertheless, these questions provide a context for future mechanistic studies that may illuminate the mechanisms by which CTLA4Ig functions to hold persistent inflammatory and other injurious responses in check.

Finally, there continues to be progress in the clinical development of belatacept. In a recent phase II trial, all renal-transplant recipient patients received peritransplant therapy with anti-CD25 (basiliximab), the immunosuppressant mycophenolate mofetil, and corticosteroids and then were randomly assigned to receive either of two dosing regimens of belatacept or the calcineurin-inhibiting drug cyclosporine (Vincenti et al., 2006). The incidence of acute rejection was similar among the three treatment groups. However, at 12 months, kidney function was significantly better in both of the belatacept treatment groups compared with the cyclosporine-treated patients. Chronic allograft nephropathy was also less common in the two groups receiving belatacept than in the group treated with cyclosporine. These results suggest that this calcineurin inhibitor-sparing regimen may prevent acute rejection as well as current therapies and do so with fewer long-term renal complications.

What Does the Future Hold?
Most importantly, the success of CTLA4Ig drug shows how breakthroughs in basic immunology are being translated into new therapies in the clinic, and raises a number of questions. Will the new agents affecting costimulatory pathways replace conventional immunosuppressive agents? There is no doubt that costimulation blockade has moved from its days as a tool for basic laboratory investigation to a proven therapy for patients with immune-mediated diseases. Opportunities for further advancement seem at hand. The success of abatacept for the treatment of rheumatoid arthritis has catalyzed its investigation as a possible therapy for systemic lupus erythematosus. Other diseases with an established therapeutic need and for which T cells have been strongly implicated in pathogenesis are also likely to receive attention. New forms of CD28-B7 blockade as well as other costimulatory inhibitors, such as anti-CD40 and CD154 antagonists (Adams et al., 2005), are on the horizon and will hopefully avoid toxicities observed with earlier drug candidates. In addition, the discovery of new costimulatory pathways will almost certainly yield more therapeutic targets for clinical testing.

Meanwhile, several important areas will require further study in patients subject to costimulatory blockade. First, will chronic costimulatory blockade have long-term consequences? When costimulatory blockade is used in combination with other immunomodulatory therapies, will it significantly increase the risk for bacterial or opportunistic infections, reactivate latent infections such as herpes zoster or tuberculosis, or reduce the efficacy of preventative vaccines? Second, CTLA4Ig therapy may deplete Tregs. Clinical studies are needed to determine the half-lives of Tregs in vivo to potentially adjust the dose of CTLA4Ig to block immune activation while preserving Treg survival (Boden et al., 2003). Finally, there is increasing evidence that CD28 is less critical for facilitating chronic immune responses (Kemball et al., 2006). Animal studies have clearly shown that CD28-B7 interactions are more important for stimulating naive T cells as opposed to activated effector or memory T cells. If so, how is abatacept suppressing inflammation in rheumatoid arthritis? Based on current dogma, the drug should not be effective if the disease is driven primarily by memory T cells, which comprise the majority of T cells in the synovial membrane of patients with rheumatoid arthritis.

We believe two approaches are needed to deal with these challenges. First, we suggest a rapid movement toward the testing of combination therapies. Based on animal studies in a variety of models, the use of CTLA4Ig combined with another costimulation antagonist or an immunosuppressive drug will be more effective than CD28 blockade alone. For example, perhaps simultaneous targeting of CD40, ICOS, LFA-1, or other cell adhesion or costimulatory pathways will induce a more robust immunomodulatory effect. Of course, this more intensive approach will require the appropriate safety precautions to minimize risks for toxicity, especially infections. Second, drug withdrawal studies are a critical next step. A relatively short course of tolerance-inducing therapy may avoid many of the toxicities associated with chronic immunosuppressive therapy, such as infection and cancer. As a community, we must press for continued development of agents, such as CTLA4Ig, that can potentially induce immune tolerance in humans and provide a deeper understanding of the mechanisms that underlie its nature.

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