The clinical utility of cytotoxic T lymphocyte antigen 4 abrogation by human antibodies

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The recent cloning and identification of a variety of regulatory and counter-regulatory molecules on T cells and antigen presenting cells has led to the development of antibodies and other molecules that either stimulate or abrogate these immune functions. Patients with autoimmune disease, graft rejection and cancer might benefit from the ability to manipulate immune regulatory pathways. The first demonstration of clinical benefit by modulation of immune regulation in cancer involves the use of human antibodies against cytotoxic T lymphocyte antigen 4. Murine preclinical studies suggested that cytotoxic T lymphocyte antigen-4 abrogation would provide clinical benefit after an antitumor vaccination. Early trials of this antibody in patients with melanoma have shown antitumor activity with and without vaccines that is associated with a state of autoimmunity. Surprisingly, the reversal of the state of autoimmunity induced by cytotoxic T lymphocyte antigen 4 antibodies by the use of corticosteroids does not eliminate clinical benefit.


Keywords: autoimmunity, human antibody, immune regulation, T cell

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Received 14 June 2006 Accepted 20 June 2006

Introduction

The explosion of information on the identity of immune regulatory molecules that provide both inhibitory and stimulatory signals to T cells has laid the groundwork for testing novel antibodies and small molecules that abrogate their signals. Cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death 1 (PD-1) and B and T lymphocyte antigen (BTLA) have been shown to be counter-regulatory molecules that attenuate T cell activity and are implicated in the induction of tolerance [1–5]. The goal for the clinical use of antibodies that abrogate T cell regulatory activity is to break tolerance to self-antigens and augment T cell activity against self-tumor antigens to achieve regression of established tumor or prevent recurrence in patients with high risk of relapse of their cancer after surgery. In the ensuing review, we will briefly describe the biochemistry of regulatory and counter-regulatory molecules on T cells, summarize some important preclinical murine and in-vitro data that focus on an important molecule, CTLA-4, and give a detailed description of the clinical experience with two human CTLA-4 antibodies. The generation of autoimmunity as a new paradigm for cancer treatment will be discussed.

Identification and characterization of the activity of cytotoxic T lymphocyte antigen 4, a T cell regulatory molecule

CTLA-4 is a regulatory molecule found on T cells that is a homologue of CD28. Its expression is up-regulated after T cell activation, can be detected within several hours and reaches a maximum after 2–3 days [1,2]. After T cell activation, most CTLA-4 is found in intracellular vesicles localized in apposition to an area of the cytoplasm close to the site of T cell receptor engagement known as the immunologic synapse. Once localized beneath the T cell–antigen presenting cell interface, CTLA-4 then translocates to the T cell surface where it carries out its inhibitory function, and has a fairly short half-life because of lysosomal targeting. CTLA-4 can compete for B7 co-stimulatory binding, inhibit interleukin (IL)-2 production as well as kinase cascades and down-regulate key components of the cell cycle machinery like cyclin-dependent kinase 4 (Cdk-4), Cdk-6 and cyclin D3 that are required for cell cycle progression [3].

Preclinical and in-vitro experiments show the importance of cytotoxic T lymphocyte antigen 4 abrogation

The regulatory role of CTLA-4 was shown by the generation of CTLA-4 knock-out mice which demonstrated that mice would die within 3–4 weeks of myocarditis and pancreatitis, with massive infiltration of lymphocytes into normal nodal and other tissue [6]. These data established the idea that CTLA-4 had an important inhibitory effect on T cell immunity. It is felt that CTLA-4 may set a T-cell signaling threshold, and that its abrogation may facilitate the expansion of
self-reactive T cells, of considerable value during the generation of tumor-specific immunity.

A variety of antibodies against CTLA-4 that abrogate its function have been tested to determine whether antitumor immunity can be augmented in their presence. Experiments with a variety of transplantable murine tumors showed that treatment with antibodies that block CTLA-4 resulted in tumor regression, even a week after tumors are established, with long-lasting immunity to rechallenge. CTLA-4 blockade enhanced immune responses to weakly immunogenic tumors [7,8]. CTLA-4 blocking antibody as a single agent, however, was not effective in causing regression of poorly immunogenic, fast growing tumors such as B16 melanoma. The failure of CTLA-4-abrogating antibodies against poorly immunogenic tumors led to its combination with other antitumor therapies. Irradiated tumor cell vaccines genetically engineered to produce granulocyte-macrophage colony-stimulating factor (GM-CSF) can induce prophylactic immunity to B16 melanoma by cross-priming [9]. The GM-CSF/B16 vaccine does not, however, impact on preexisting established tumors. When the cell vaccine was combined with anti-CTLA-4 antibody, B16 tumors completely regressed when vaccination was initiated 4 days after tumor initiation and tumor size significantly decreased even when vaccination was delayed for 1 week [10].

Regression of B16 melanoma after immunization with a GM-CSF-transduced tumor cell vaccine with anti-CTLA-4 antibody was accompanied by vitiligo characterized by the presence of inflammatory cells and disappearance of melanocytes in affected areas of the skin [11]. Depigmentation was not observed in mice immunized with a GM-CSF/B16 vaccine alone. This depigmentation suggested that the antitumor response was at least in part directed against differentiation-related self-antigens, and suggested that CTLA-4 blockade induced breaking of peripheral T cell tolerance [12]. Generation of prophylactic responses by the GM-CSF-transduced cell vaccine did not depend on CD8+ T cell activity, but did require CD4+ T cells in the above experiments. This suggested that cross-priming resulted in the induction of CD4+ T cells with specificity for major histocompatibility complex class II-restricted peptides derived from the B16 tumor cells which themselves were known to be class II negative, and that tumor was eliminated by other effector cells [13]. In contrast, rejection of established tumors by the GM-CSF/B16 vaccine in combination with anti-CTLA-4 was independent of CD4+ T cells. CD8+ T cells and cells expressing NK1.1 were required for rejection of established tumor, as were perforin and Fas–Fas ligand. These data indicate that CTLA-4 blockade directly induced cytotoxic CD8+ T lymphocytes in the absence of T cell help. The mechanism of immune activation and subsequent tumor regression may have lowered the threshold of costimulatory signals needed for activation and differentiation of CD8+ T cells to a level that did not require CD4+ T cell-mediated help via CD40 ligand. Alternatively, CTLA-4 blockade may have expanded a critical number of tumor-reactive T cells needed to mediate tumor rejection. One possibility is that effector CD8+ T cells may be generated in situations in which CD4+ T cells are absent or ineffective. CTLA-4 antibody was also found to be effective in mediating regression together with a GM-CSF-transduced cell vaccine in a spontaneously arising prostate cancer model [14], and could eliminate recurrence in a model of resected cancer with the same spontaneously arising tumor at risk of relapse [15]. Abrogation of CTLA-4 was also found to augment the effects of suboptimal doses of chemotherapy in a transplanted murine tumor model [16], further supporting the antitumor role of CTLA-4 antibodies and suggesting a possible additive or even synergistic role with chemotherapy.

In the prior experiments in mice, CD4+ T cells were not required for tumor rejection after combined vaccination and CTLA-4 abrogation [17], but tumor rejection and depigmentation were actually enhanced in their absence. This suggested that T regulatory cells might play a role in modulating responses to self-differentiation antigens. Consistent with this hypothesis, depletion of CD25+ cells before vaccine and CTLA-4 abrogation therapy increased its effectiveness, allowing the rejection of larger tumor burdens [18]. This increase in antitumor efficacy was associated with an increase in the number of melanoma antigen-specific T cells. These findings suggested that T regulatory cells may have interfered with the induction of antitumor immunity and should be factored into the development of tumor immunotherapy treatments that include CTLA-4 abrogation. CTLA-4, however, did not appear to mediate suppression by T regulatory cells, and T regulatory cells and CTLA-4 may represent two independent mechanisms involved in down-modulating T cell responses.

Clinical experience with two different cytotoxic T lymphocyte antigen 4 abrogating molecules

Single dose phase I trials of two different anti-CTLA-4 antibodies have been conducted. Patients with metastatic melanoma and ovarian cancer were treated with single doses of MDX-010 or Ipilimumab from Medarex at doses from 1 to 3 mg/kg [19,20]. Two patients of 17 treated had a partial response, and both the responders had melanoma and had previously been vaccinated with a genetically transduced cell vaccine. Interestingly, of the six patients with ovarian cancer, there were no objective responders, but all six had evidence of tumor necrosis after resection.
of accessible lesions. One patient developed rash, and one pruritis, neither of which was dose limiting after a single dose. The antibody was then tested at a fixed dose of 3 mg/kg every 3 weeks with a multi-peptide gp100 vaccine that included Montanide ISA 51, an oil-based adjuvant, in patients with refractory metastatic melanoma [21]. Fifty-six patients were treated with up to four doses every 3 weeks. Seven responses were seen in that trial, five partial responses (PR) and two complete responses (CR). Five of the responses were sustained beyond 25 months and are ongoing. Of 14 there were 5 responders in patients with grade III or more autoimmunity, compared with two responses in 42 patients without autoimmune side effects, with a P < 0.008 for the association between autoimmunity and clinical response. The autoimmune manifestation observed in that trial was named immune breakthrough event (IBE).

Ipilimumab has also been studied in several studies as an adjuvant for a multi-peptide gp100, Melan-A and tyrosinase vaccine in patients with resected high-risk stages III and IV melanoma [22]. In the first trial, 19 patients received escalating doses of antibody from 0.3 to 1 to 3 mg/kg every 4 weeks for six doses, then two more doses at a 3-month interval. In a follow-up trial, 25 additional patients received seven doses of Ipilimumab at 3 mg/kg every 8 weeks with the same vaccine given 12 times in 12 months. Dose limiting toxicity consisting of IBEs similar to those observed in the trial in metastatic disease was observed in three out of five patients at 3 mg/kg every 4 weeks in the first trial, so the second trial was performed with antibody at 3 mg/kg but given every 8 weeks to lessen the toxicity. For 22 patients with an IBE, there were five relapses, versus 14 of 22 without an IBE. For all 44 patients in the two trials, there was an association between immune breakthrough events and time to relapse, with P < 0.01. Immune responses to the three melanoma antigens were observed in most patients on both trials, but not clearly different from prior trials without CTLA-4 abrogation. A similar spectrum of autoimmunity was seen as in the trials of Ipilimumab in unresectable stage IV melanoma. These data support and extend the idea that IBEs are associated with clinical benefit after CTLA-4 abrogation, and suggest that the antitumor activity of Ipilimumab is clearly associated with and may depend on the autoimmune effects [23].

Anti-CTLA-4 antibody CP-675,206 from Pfizer (New London, Connecticut, USA), or Ticilimumab, has been tested in a single dose phase I trial at doses from 0.01 to 15 mg/kg [24]. Thirty-nine patients were treated, of whom 34 had melanoma. Four objective responses were noted with two CR and two PR of 29 evaluable melanoma patients (13%). All four responses were sustained for more than 25 months. Three of 12 responses had autoimmune side effects, compared with one of 18 without autoimmunity, similar to that seen in the trials of Ipilimumab alone or with vaccine. Three of six dose limiting toxicities were observed at the 15 mg/kg dose, so the dose chosen for a phase II testing was 10 mg/kg.

Ipilimumab has also been combined with high dose IL-2 and with Dacarbazine, both FDA approved agents for melanoma [25,26]. A small randomized trial of Ipilimumab alone or with Dacarbazine at 250 mg/m^2 on days 1–5 given every 3 weeks was conducted with preliminary results presented at the American Society for Clinical Oncology meeting in 2005 [25]. For 35 patients who received the combination, there were six objective responses for a 17% response rate, with a median survival of 14.2 months; for 37 patients who received Ipilimumab alone at 3 mg/kg every 3 weeks, the response rate was only 5% with a median survival of 11.2 months. The favorable survival results of the combination therapy have led to a large phase III randomized trial of Dacarbazine with placebo compared with Dacarbazine plus Ipilimumab at 10 mg/kg for patients with previously untreated stage IV melanoma.

Ipilimumab was tested in an escalating dose trial with IL-2 at the National Cancer Institute (NCI) standard dose of 720,000 IU/kg given intravenously every 8 h [26]. Thirty-six patients were treated with Ipilimumab doses from 0.3 to 3 mg/kg. Eight responses were found, with three CR and five PR. Twenty-four patients received two cycles of IL-2 with antibody at 3 mg/kg four times. Five of 24 patients had objective responses, all with autoimmune side effects, and the IBE rate of five of 24 seemed no different from prior trials of the antibody at that dose, indicating that the IL-2 might have additive benefit clinically but no clear alteration in the IBE rate. This combination deserves to be taken further in a phase II study.

Clinical benefit correlates with autoimmune side effects after cytotoxic T lymphocyte antigen 4 abrogation

The extensive published experience with groups at NCI and University of Southern California using Ipilimumab and data from a published phase I dose escalating trial of Ticilimumab indicate that similar dose limiting autoimmune phenomena that have been termed ‘immune breakthrough events’ or IBEs have been observed with the use of both antibodies [23,24]. The principal side effects of these antibodies have been diarrhea, colitis, hypophysitis and skin reactions. The spectrum of gastrointestinal side effects can range from several loose bowel movements daily to bloody diarrhea and colonic perforation. At the NCI, the incidence of colitis was summarized in 193 patients with melanoma and renal cancer who received Ipilimumab alone or with a vaccine [27]. Forty one of those patients, or nearly 21%, had
evidence of grade III or greater diarrhea or colitis. Thirty six were colonoscoped, with findings of diffuse ulcerations and biopsy-proven colitis. The onset of symptoms occurred a median of 11 days after the last infusion of antibody. Most patients recovered with the use of intravenous and oral steroids in tapering doses, sometimes requiring long durations of 1–2 months. Often uveitis and/or photophobia was associated with the gastrointestinal findings, as was fever [28]. Ulcerations could be seen in the pharynx, stomach and small bowel as well as the colon or rectum. Four patients in that cohort were resistant to steroid tapers and received Remicade (Infliximab), a chimeric antitumor necrosis factor blocking antibody, with relief of symptoms. Four patients had perforations, of which three had renal cell carcinoma. The mortality rate of those with gastrointestinal toxicity was 5%. A striking association of gastrointestinal autoimmune manifestations with clinical response in metastatic disease, and with time to relapse in patients with high-risk resected melanoma was observed [21,22].

The same group from NCI also reported on the incidence of hypophysitis with Ipilimumab, which was documented in eight of 163 cases, approximately 5% [29]. This condition, which is rarely reported post-partum, presented with edema and heterogenous enhancement of the pituitary on magnetic resonance imaging, and low adrenocorticotropic hormone and/or thyroid stimulating hormone as well as cortisol. Patients required replacement with corticosteroids and/or mineralocorticoids as well as thyroid hormones depending on which axis of the pituitary was affected. As with colitis, the incidence of hypophysitis as an IBE was associated with clinical response, of the eight patients described with hypophysitis, five had a clinical response with $P < 0.008$ for the association. Long-term hormone replacement has been required in virtually all patients with this form of IBE.

Uveitis as an IBE has been found to be associated with colitis. In a study of 40 patients with colitis, the incidence of uveitis was significant, with inflammatory cells in the anterior chamber, and photophobia as common manifestations [28]. The condition was successfully treated in all cases with prolonged administration of steroid topical drops.

The immediate use of high-dose intravenous steroids, followed by a tapering dose of oral prednisone, has been found to induce recovery in most cases of colitis and other grade III or above toxicity. Budesonide or Entocort, an orally administered and poorly absorbed steroid, has been used in patients with grade II diarrhea, and is currently being tested in a placebo-controlled randomized phase II trial with reduction of colitis and diarrhea as endpoints.

**Mechanism of action of cytotoxic T lymphocyte antigen 4 abrogating antibodies**

The mechanism by which CTLA-4 abrogation induces tumor regression and protects against relapse appears to be linked to the ability to induce autoimmunity as manifested by IBEs. An attractive hypothesis is that T regulatory cells, which are CD4+ , CD25+ cells that express the marker FoxP3 and are CTLA-4 positive, may be suppressed or eliminated by CTLA-4 abrogation, unleashing antitumor immune effectors and also causing ‘bystander’ damage to the gastrointestinal tract and other organs manifested as autoimmune colitis and other IBEs. Little evidence exists to favor this hypothesis, however, because work by the NCI group has shown no evidence of any effect of CTLA-4 antibodies on T cell regulatory activity in *vitro* or *in vivo* using peripheral blood cells from patients receiving Ipilimumab [30]. No phenotypic changes in numbers of CD4+CD25+ regulatory cells were observed in either of the early trials of Ipilimumab with vaccines [19–22]. Finally, the murine data do not support the idea that regulatory activity is required for the antitumor activity of CTLA-4 abrogating antibodies [31,32]. In contrast, objective responses in melanoma patients who received Ticilimumab were associated with reductions in regulatory T cells and constitutive high levels of IL-10, detection of increased non-specific IL-2 secretion and a positive correlation between CTLA-4 and glucocorticoid-induced tumor necrosis factor receptor detected by reverse-transcriptase polymerase chain reaction [33]. Non-responders had stable levels of T regulatory cells and high IL-10 secretion, and a positive correlation between CTLA-4 and PD-1. These data are consistent with the idea that CTLA-4 abrogation might alter T regulatory cell activity. An alternative idea is that CTLA-4 antibodies directly impact on the activity of effector cells, promoting both tumor-specific and non-specific T cells with low avidity for self-antigens. Some of the clues to favor this idea are the reproducible increase in activated CD4+/HLA-DR+ T cells observed with both Ipilimumab and Ticilimumab treatment, and the evidence that trafficking molecules such as CCR4 are altered on T cells in patients treated with Ipilimumab [22].

**Conclusion**

CTLA-4 abrogating antibodies are the first in a class of antibodies that alter T cell function by either abrogating inhibitory influences or potentiating their activation. An attractive feature of these antibodies is the induction of long-term, sustained clinical responses in those who are refractory to prior therapy. The proportion of objective responders is modest, in the range of 10–20%, but an unknown number of patients with stable disease have apparently never progressed. It is not clear that optimal dosing and scheduling of this interesting drug has been achieved. It is certainly not clear what immunomodulators and cytotoxics would be best to combine with CTLA-4.
abrogating antibodies. It does seem clear that the only useful surrogate marker for clinical benefit is the development of autoimmune side effects, which clearly are associated with objective response, survival and time to relapse. A number of CTLA-4 and PD-1 polymorphisms were found whose presence may predict response and autoimmunity. The ability to predict whether side effects will be severe or will have a rapid onset may be useful to limit the impact of IBEs, and concepts such as the use of preventive Budesonide, low dose corticosteroids and other agents that may paradoxically increase T regulatory activity are worth pursuing. Ongoing murine and human studies to elucidate the mechanism of antitumor activity of CTLA-4 abrogation will hopefully facilitate the use of these promising agents with minimal toxicity.

**Acknowledgements**

The author wishes to thank Drs Jim Allison, Steven Rosenberg, Toni Ribas, James Yang and Luis Camacho for helpful discussions.

**References**


