Anti–CTL-Associated Antigen 4: Are Regulatory T Cells a Target?

□□ *Commentary on O'Mahony et al., p. 958*

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In this issue, O'Mahony et al. (1) targeted CTL–associated antigen 4 (CTLA-4) in previously vaccinated solid tumor patients using the human anti–CTLA-4 antibody ipilimumab (MDX-010). Ipilimumab, together with a second human anti–CTLA-4 antibody, ticilimumab, are currently in phase 3 clinical trials in a number of cancers. The study was a single center, open-label pilot phase II trial consisting of 11 patients, 4 with non–Hodgkin's lymphoma immunized to tumor-specific idiotype, 4 with prostate cancer immunized using a poxvirus vector encoding prostate-specific antigen, and 3 with colon cancer immunized using a mutated ras peptide–pulsed dendritic cell vaccine, all of whom failed tumor-specific vaccination therapy. Patients received four cycles of ipilimumab, an initial dose of 3 mg/kg i.v. followed by three monthly doses of 1.5 mg/kg. The primary end point of the study was to assess ipilimumab toxicity at the specified dosing schedule. Overall, ipilimumab was well tolerated with a number of grade 1 and 2 toxicities possibly related to treatment. Patients with more advanced metastatic disease manifested grade 3 and 4 toxicities likely attributable to their advanced disease rather than ipilimumab treatment. One patient with advanced metastatic colon cancer died within 30 days of treatment due to disease progression. Tumor responses were limited to two patients with non–Hodgkin's lymphoma who experienced limited tumor regression at selected metastatic sites. No objective tumor responses were seen in the patients with prostate or colon cancer.

In addition to evaluating ipilimumab toxicity and tumor response, the secondary end points of the study were aimed at elucidating the effects of anti–CTLA-4 on relevant immune variables. These included the effects of ipilimumab on the number of peripheral CD4+CD25+ regulatory T cells (Tregs) and the activation of peripheral vaccine-specific CD8+ effector T cells.

CTLA-4 Regulates Immune Responses

The adaptive immune response requires two signals between the antigen-presenting cells (APC) and the effector T cell as shown in Fig. 1. The primary signal is mediated by the T cell receptor and the specific antigenic peptide presented in the context of MHC class I or class II molecules expressed on the APC surface, whereas the secondary signal is mediated through constitutively expressed costimulatory molecules on the T cell (CD28) and the APC (B7.1/CD80 or B7.2/CD86). The presence of both signals triggers intracellular events resulting in the activation and interleukin (IL)-2–dependent clonal proliferation of T cells expressing T cell receptor specific for the presented antigen. Unchecked, T cell proliferation can lead to autoimmunity and even death. The immune system has evolved homeostatic mechanisms that down-regulate this clonal expansion, which include the up-regulation of CTLA-4 (2). Activated T cells up-regulate surface CTLA-4, which has a higher affinity for B7.1 and B7.2 than CD28, effectively competing for B7.1 and B7.2 binding and inducing inhibitory signals in effector T cells. This leads to the dampening of the effector T cell response.

In addition to well-documented tumor escape mechanisms such as the down-regulation of surface MHC class I molecules and cytokine-mediated reduced responsiveness to tumor-associated antigens (TAA), the findings in both preclinical and clinical studies that treatment with anti–CTLA-4 is associated with enhanced T cell responses and antitumor activity have validated it as a target for immunotherapy (3–6). In addition to activity seen when used as a single agent, preclinical studies combining anti–CTLA-4 with vaccine (7), and reports of enhanced immune and tumor responses in patients previously treated with vaccine support its use following vaccine treatment (8).

O'Mahony et al. discuss two general mechanisms by which CTLA-4 blockade may generate antitumor responses. The first is mediated through CTLA-4 blockade on tumor-specific effector T cells (CTL) discussed above, resulting in an increased propensity for clonal activation and expansion. Consistent with this mechanism, Maker et al. recently provided evidence that treatment with ipilimumab in patients with metastatic melanoma or metastatic renal cancer increased the numbers of activated effector cells among peripheral blood mononuclear cells (9). The second possible mechanism is mediated through CTLA-4 targeted depletion of tumor-induced Tregs, which act to inhibit immune responses against TAA. Depletion of Tregs is proposed to overcome nonresponsiveness to TAA and allow the generation of tumor-specific responses. This is the hypothesis favored by O'Mahony et al. and forms the basis for the discussion of CTLA-4 in the context of tumor-associated Tregs.

CTLA-4 and Tregs in Tumor Immunity

Tregs are a suppressive CD4+ T cell population that express high levels of surface CD25 (the high affinity IL-2α receptor subunit), CTLA-4, and the glucocorticoid-induced tumor

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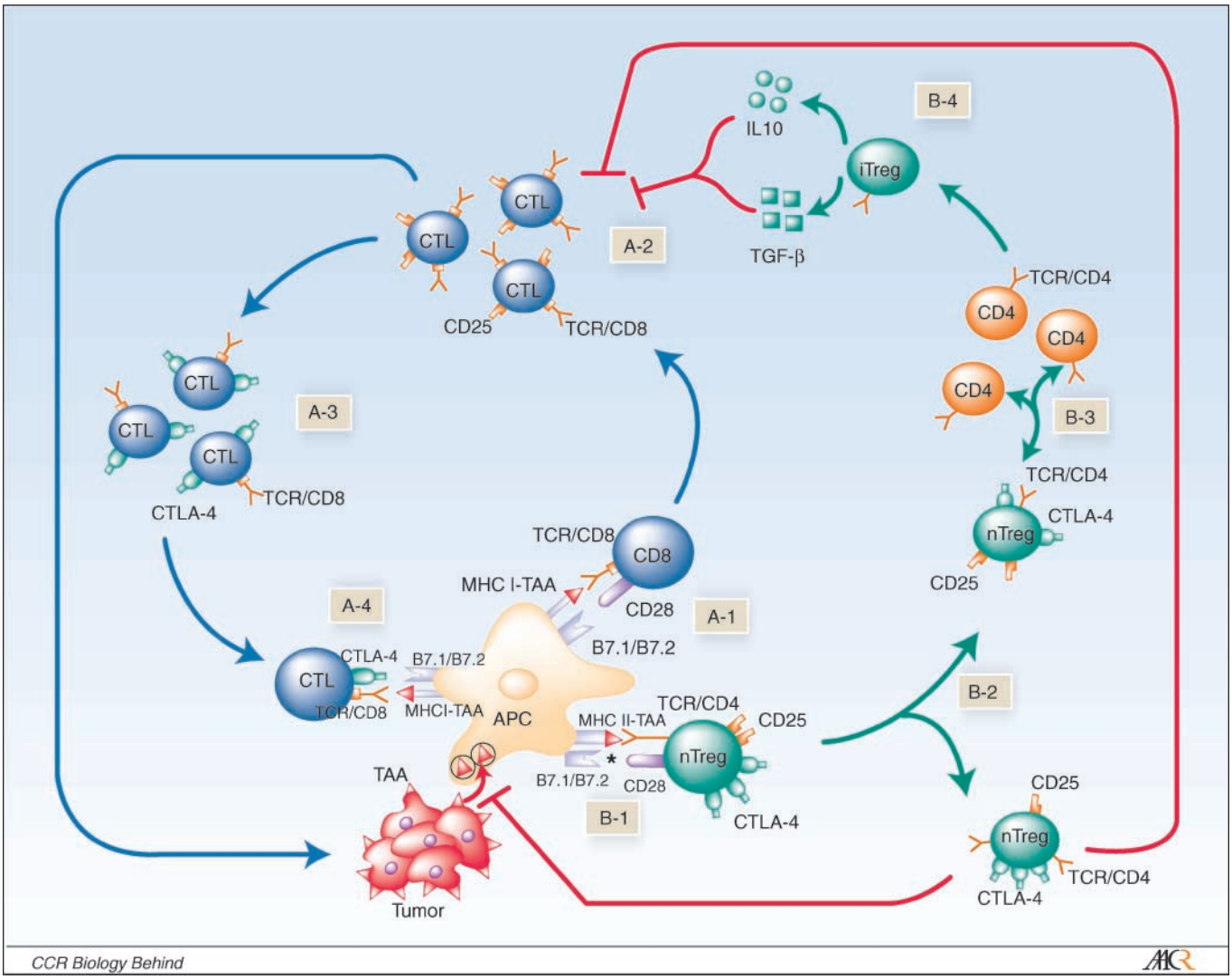

Fig. 1. Functions of CTLA-4 in tumor immunity. APC presentation of TAA in complex with MHC I to CD8+ T cells (*A-1*) triggers activation and clonal expansion of tumor-specific effectors (CTL) mediated by increased IL-2 production and increased surface IL-2 receptor α (CD25) expression (*A-2*). Tumor-specific CTL can recognize TAA on tumor resulting in tumor lysis and/or cytokine production. Up-regulation of surface CTLA-4 follows clonal expansion to regulate the immune response (*A-3*). When CTLA-4 encounters B7.1/B7.2 on APC, it binds with greater affinity than does CD28, resulting in intracellular down-regulatory signals within tumor-specific CTL (*A-4*). TAA can also be presented to CD4+CD25+ Tregs (*B-1*), which constitutively express high levels of surface CD25 and CTLA-4, resulting in activation of tumor-specific Tregs (*B-2*). Treg activation is antigen-specific and requires interaction between T cell receptor/CD4 and TAA in the context of MHC II, but may not require costimulation (*B-1**). Treg suppression of antitumor responses is not antigen-specific and occurs through two possible pathways (*B-2*). The first pathway is mediated by direct contact, likely involving surface CTLA-4, natural Treg inhibition of TAA presentation by APC, and cytotoxic killing by tumor-specific CTL. The second pathway is mediated by the generation of induced Tregs (*iTregs*), which are CD4+CD25− T cells converted to a Treg phenotype by signals from natural Tregs (*B-3*). Converted induced Tregs are associated with the production of immunosuppressive cytokines, including IL-10 and transforming growth factor-β (*B-4*), which suppress T cell responses, specifically, the generation of tumor-specific CTL.

necrosis factor-α receptor (GITR), which when activated, abrogates Treg function (10). Tregs have also been shown to produce immunosuppressive cytokines such as IL-10 and transforming growth factor-β (11). Tregs are primarily identified by intracellular expression of the forkhead/winged-helix transcriptional factor Foxp3, critical for Treg development (12). Tregs can be further classified into natural Tregs and induced Tregs, which differ in their origin/development, requirements for activation with regards to costimulation (natural Tregs may not require costimulation unlike induced Tregs), and potential mechanisms of action (13). Whereas activation of Tregs is considered antigen-specific, their immunosuppressive function is nonspecific, inhibiting multiple phases and events in the immune response from antigen presentation to effector functions through a variety of identified mechanisms (14). Among these mechanisms is up-regulation of CTLA-4 on the surface of Tregs (see Fig. 1), which through cell-to-cell contact, can suppress the activation and expansion of effector cells specific for both normal self and tumor antigens (15, 16). Therefore, whereas CTLA-4–expressing Tregs may play a critical role in maintaining self tolerance, they may also facilitate a level of nonresponsiveness to tumor antigens.

Tregs have been shown to be present in tumor and tumor-draining lymph nodes in animal models (17), and coexist with primed effector T cells in these compartments (18). This presence of Tregs in tumor-draining lymph nodes and tumor provides a potential inhibitory population blocking or "balancing"

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effector cell function. Thus, depletion of Tregs or blockade of Treg function using targeted antibodies has the potential to remove Treg suppression and enhance antitumor immunity. O'Mahony et al. provide evidence that treatment of patients with ipilimumab has the potential to result in antitumor responses via Treg depletion or blockade, as well as through the direct activation of tumor-specific CD8+ CTL.

Although the limited data set provided failed to show the effects of ipilimumab on tumor antigen – specific CD8 responses, ipilimumab was shown to modulate numbers of Tregs in the periphery. Peripheral blood mononuclear cells were stained for CD4, CD25, and CD62L (or surface L-selectin, which has been identified as a marker of more potent Treg suppression activity) before and after ipilimumab treatment. The number of CD4+CD25+CD62+ Tregs showed a bimodal pattern following treatment. Tregs initially decreased after ipilimumab dosing (within 3 days) by approximately 1/5 to 1/3 of pretreatment levels. Following the third day posttreatment, the number of Tregs approached pretreatment levels, and within 4 weeks (or immediately prior to the next ipilimumab cycle), had returned to or exceeded pretreatment levels. Further analysis was done on one of the non – Hodgkin's lymphoma patient responders using reverse transcription-PCR for Foxp3 mRNA expression among peripheral blood mononuclear cells. Foxp3 mRNA expression showed a similar bimodal pattern to Treg surface marker expression. Previous data from animal models showed similar results of rebounding Tregs within several days following anti-CD25 antibody (19). Kohm et al. recently provided evidence that rather than resulting in Treg depletion, the effects of systemically administered anti-CD25 may be the result of a functional blockade of Tregs mediated by antibody-induced shedding of surface CD25 as opposed to Treg depletion, which has yet to be definitively established (20). Whether anti – CTLA-4 acts through functional blockade of CTLA-4 on effector T cells as opposed to or in addition to depletion of CTLA-4 – expressing Tregs remains unresolved. In any case, the data presented by O'Mahony et al. suggests that ipilimumab is capable of blocking Treg function in the peripheral blood and supports the hypothesis that anti – CTLA-4 therapy might affect antitumor immunity through multiple mechanisms including Treg depletion or blockade. The authors propose that the short-lived early effects on Treg function posttreatment may have been sufficient to have allowed the generation of antitumor immune responses, even though their limited data failed to document such an enhancement.

The Clinical Potential for Anti – CTLA-4 – Targeted Therapy

O'Mahony et al. provided evidence that ipilimumab decreases the number of Tregs in peripheral blood mononuclear cells, which may correlate with tumor regression. Although the authors did not observe differences in peripheral CD8+ CTL responders as measured by tumor-specific IFN-γ production, this does not eliminate the possibility that tumor-specific effectors were present in the local tumor environment or tumor-draining lymph node. Yang et al. and others have shown in animal models that tumor-specific CTL could be generated in the tumor-draining lymph node of untreated tumor-bearing mice, but that responders were sequestered to the local tumor environment and were not detected in the spleen or peripheral blood (21). Furthermore, Quezada et al. showed in tumor-bearing mice that anti – CTLA-4 antibody in the presence of a recombinant granulocyte macrophage colony-stimulating factor producing vaccine (Gvax) could increase the number of tumor-specific responders in the tumor microenvironment, altering the balance of primed effectors to Tregs, which allowed for an effective antitumor response (22). In this study, O'Mahony et al. reported the presence of increased infiltrates of CD3+CD4+ and CD3+CD8+ T cells into the lymph nodes of one non – Hodgkin's lymphoma patient following ipilimumab treatment. Although this may be consistent with the hypothesized mechanism of action of ipilimumab, without a larger number of patients, this remains speculative.

It should be noted that patients enrolled on the O'Mahony et al. study all had advanced disease and that each patient had failed tumor-specific vaccination likely due both to the extent or bulk of disease as well as the potential activity of one or a number of immune escape mechanisms. Recent preclinical studies from the Levitsky group have also shown that vaccines encoding tumor antigens, given in the context of Treg cell activation, may actually enhance the activity and/or numbers of Tregs and thus further inhibit tumor-specific effector function (23). These findings support the view that tumor vaccines are likely to be more effective when given in the minimal residual disease state in which both tumor burden and tumor-associated immune dysfunction may be at a minimum.

In summary, there is increasing evidence that anti – CTLA-4 immunotherapy has potential clinical benefit. Preclinical studies, and increasingly, clinical trials have shown antitumor activity. Given as a single agent or in combination with vaccine, preclinical studies clearly show the efficacy of anti – CTLA-4 treatment at enhancing antitumor T cell responses and tumor regression. Clinical studies have shown similar single agent responses with enticing data showing possible enhanced activity in patients previously treated with vaccine. There is substantially less data available on the effects of modulating Treg populations. Although the preclinical support for such manipulation is clear, clinical studies are at the preliminary stage of associating Treg numbers with tumor responses. Whereas there have been a small number of studies reporting the use of the agent Ontak, a fusion molecule between the ligand of the CD25 receptor and a toxin, to diminish Treg function in patients, they are extremely limited and show conflicting results (24, 25). Until there is validation of an agent or regimen that functionally inhibits Tregs in man, the role of Tregs in clinical cancer remains unclear. The findings of O'Mahony et al., which show an apparent reduction in cells positive for Treg markers using ipilimumab, provides support that such modulation may be possible. It should be stressed, however, that given the small number of patients studied and the limited immune data, that although the O'Mahony hypothesis is intriguing, it remains to be proven until appropriately powered clinical trials are carried out.

Acknowledgments

We apologize to our colleagues for not citing many important publications. Due to the limitation in space and the numbers of references, this was not possible.

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References

1. O'Mahony D, Morris JC, Quinn C, et al. A pilot study of CTLA-4 blockade after cancer vaccine failure in patients with advanced malignancy. Clin Cancer Res 2007;13:958–64.
2. Brunet JF, Denizot F, Luciani MF, et al. A new member of the immunoglobulin superfamily-CTLA-4. Nature 1987;328:267–70.
3. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996;271:1734–6.
4. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol 2001;19:565–94.
5. Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 2005;23:6043–53.
6. Thompson RH, Allison JP, Kwon ED. Anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) immunotherapy for the treatment of prostate cancer. Urol Oncol 2006;24:442–7.
7. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med 1999;190:355–66.
8. Hodi FS, Mihm MC, Soiffer RJ, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A 2003;100:4712–7.
9. Maker AV, Attia P, Rosenberg SA. Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. J Immunol 2005;175:7746–54.
10. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. Nat Immunol 2002;3:135–42.
11. Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25− cells to develop suppressive activity: the role of IL-2, TGF-β, and IL-10. J Immunol 2004;172:5213–21.
12. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057–61.
13. Chattopadhyay S, Chakraborty NG, Mukherji B. Regulatory T cells and tumor immunity. Cancer Immunol Immunother 2005;54:1153–61.
14. von Boehmer H. Mechanisms of suppression by suppressor T cells. Nat Immunol 2005;6:338–44.
15. Takahashi T, Tagami T, Yamazaki S, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000;192:303–10.
16. Sansom DM, Walker LS. The role of CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) in regulatory T-cell biology. Immunol Rev 2006;212:131–48.
17. Yamaguchi T, Sakaguchi S. Regulatory T cells in immune surveillance and treatment of cancer. Semin Cancer Biol 2006;16:115–23.
18. Hiura T, Kagamu H, Miura S, et al. Both regulatory T cells and antitumor effector T cells are primed in the same draining lymph nodes during tumor progression. J Immunol 2005;175:5058–66.
19. Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. Tumor rejection by *in vivo* administration of anti-CD25 (interleukin-2 receptor α) monoclonal antibody. Cancer Res 1999;59:3128–33.
20. Kohm AP, McMahon JS, Podojil JR, et al. Cutting edge: anti-CD25 monoclonal antibody injection results in the functional inactivation, not depletion, of CD4+CD25+ Tregulatory cells. J Immunol 2006;176:3301–5.
21. Yang AS, Monken CE, Lattime EC. Intratumoral vaccination with vaccinia-expressed tumor antigen and granulocyte macrophage colony-stimulating factor overcomes immunological ignorance to tumor antigen. Cancer Res 2003;63:6956–61.
22. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 2006;116:1935–45.
23. Zhou G, Drake CG, Levitsky HI. Amplification of tumor-specific regulatory T cells following therapeutic cancer vaccines. Blood 2006;107:628–36.
24. Dannull J, Su Z, Rizzieri D, et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients and depletion of regulatory T cells. J Clin Invest 2005;115:3623–33.
25. Attia P, Maker AV, Haworth LR, Rogers-Freezer L, Rosenberg SA. Inability of a fusion protein of IL-2 and diphtheria toxin (Denileukin Diftitoxin DAB389IL-2, ONTAK) to eliminate regulatory T lymphocytes in patients with melanoma. J Immunotherapy 2005;28:582–92.

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