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How Does an Anti-CTLA4 Antibody Promote Cancer Immunity?

Yang Liu and Pan Zheng

Division of Immunotherapy, Institute of Human Virology and Department of Surgery, University of Maryland Baltimore School of Medicine, Baltimore, MD21201, USA

Abstract

Anti-CTLA-4 antibodies can induce lasting protection for some melanoma patients. However, their therapeutic potential is limited by significant immunotherapy-related adverse effects (irAE). Here, we argue that the therapeutic effect may be based on an agonist activity that is fundamentally distinct, and can be therapeutically differentiated, from the antagonist activity responsible for irAE.

Reappraising the CTLA-4 Checkpoint Blockade Hypothesis?

Ipilimumab, a monoclonal antibody against human T cell protein CTLA-4 constitutes the first of immune cell-targeting antibodies approved by US Food and Drug Administration for cancer immunotherapy [1]. Long-term follow-up of anti-CTLA-4 antibody-treated patients has revealed that Ipilimumab has the extraordinary potential to induce long-term protection against cancer relapse [2]. This has been based on the assumption that inhibiting naïve T cell-intrinsic negative regulatory signaling from B7-CTLA-4 interactions, or immune checkpoint blockade [3], can potentiate the antitumor activity of T cells. However, recent studies from us and others have called for a reappraisal of the anti-CTLA-4 checkpoint blockade hypothesis [4-7]. To address this question, we first provide a historical context on CTLA-4 checkpoint blockade, assessing the clinical benefits and challenges of this cancer treatment. We then discuss recent studies from independent groups challenging the hypothesis of checkpoint blockade, and examine how these new insights might potentially contribute to improving the next generation of safer and more effective anti-CTLA-4 immunotherapies. asl

CTLA-4 Function and Cancer Immunotherapy

CTLA-4 has been termed a negative regulator of naïve T cell activation because intact and Fab antibodies directed against CTLA-4 can stimulate T cell activation [8], and because global inactivation of *Ctla4* in mice [9] and in humans [10] induces lymphoproliferative autoimmune diseases. This concept provided a theoretical basis for developing anti-CTLA-4 antibodies for cancer immunotherapy. However, later studies reported that naïve T cells do not express detectable CTLA-4, and that *Ctla4* is a target gene of transcription factor Foxp3 [11], and is thus expressed predominantly on regulatory T cells (Treg(s)). importantly,

lineage-specific deletion of mouse *Ctla4* in Tregs alone is sufficient to largely recapitulate the fatal lymphoproliferative diseases associated with germline mutations in the *Ctla4* gene [12], which argues that CTLA-4 primarily functions in Tregs. Moreover, since *Ctla4*-deficient Tregs are defective in suppressing mouse and human effector T cells [10, 12], we posit that CTLA-4 should be considered as a cell-intrinsic positive regulator -- rather than negative regulator -- of Treg function. It follows that if the functions of CTLA-4 are called for reinterpretation, then so should the immunotherapeutic effects of anti-CTLA-4 antibodies.

Selective Depletion of Regulatory T cells in Tumors is Necessary for the Therapeutic Effect of Anti- CTLA-4 Antibodies

Since CTLA-4 regulation of immune activation occurs primarily through its positive effect on Tregs (which can negatively regulate antitumor immunity) [13], the therapeutic activities of anti-CTLA-4 antibodies may be based on their effects on Tregs; either by blocking the function of CTLA-4 on Tregs or by inducing the elimination of Tregs, given that the immunosuppressive effect of Tregs, is presumed to be a major impediment for effective anti-cancer immunity [14]. Indeed, several lines of evidence have demonstrated that selective deletion of Tregs in the tumor microenvironment is necessary for the therapeutic effect of anti-CTLA-4 antibodies. In one report, various antibodies harboring different IgG isotypes each, were engineered from the anti-mouse CTLA-4 antibody 9D9 [4]. While these antibodies were anticipated to be equally effective in blocking CTLA-4 function in vivo, they differentially depleted Tregs within the tumor microenvironment. Thus, while a non-depleting 9D9-IgG1 had no effect on tumor growth, depleting 9D9-IgG2a or IgG2b, which induce significant and selective Treg depletion in tumor microenvironment but not in the periphery lymphoid organ, induce tumor rejection. Moreover, flow cytometry data showed that the selective depletion within the tumor microenvironment occurred for the $\text{Foxp3}^+\text{CTLA-4}^+$ Treg subset, but not for $\text{Foxp3}^-\text{CTLA-4}^+$ T cells, ruling out depleting effector T cells as potential mechanism [7]. Selective Treg depletion can be explained by the higher expression of antibody target CTLA-4 that was observed on tumor infiltrating Tregs when compared to either Tregs in the periphery or non-Tregs within tumor microenvironment [4, 7]. Consistent with this notion, two independent laboratories demonstrated that activating the **FcR** was necessary to achieve the anti-tumor effect of anti-CTLA4 antibodies in multiple tumor models [5, 6]. Accordingly, using human CTLA-4 knockin mice and anti-mouse FcR antibodies, we demonstrated that FcR-mediated Treg deletion was necessary for the effector function of Ipilimumab, the anti-human CTLA-4 antibody [7]. The requirement for the correct isotype and FcR interaction has now been extended into a human FcR system using humanized mice that express various human FcRs [15].

Another study reported that two anti-human CTLA4 antibodies, (Ipilimumab; IgG1 isotype) (tremelimumab, IgG2), increased the infiltration of intratumoral CD4⁺ and CD8⁺ T cells in melanoma tissues when compared with tissues from untreated patients, without causing a significant reduction in the total number of Tregs [16]. Of note, unlike tremelimumab, ipilimumab exhibited a more efficient Treg-depleting activity in the tumor, leading to a

reduction in Treg frequency when the total number of tumor-infiltrating T cells were taken into consideration [16]. It is intriguing whether the reduction of Treg frequency by Ipilimumab explain its apparently superior therapeutic effect.

Checkpoint blockade May Be Unnecessary for the Effector Function of Anti-CTLA-4 Antibodies

While the above studies have demonstrated the critical contribution of Treg-depletion in achieving an immunotherapeutic effect in mouse tumor models, they did not address whether CTLA-4 checkpoint blockade contributed to the therapeutic effect. To fill this gap, we recently reported evidence that argues against the need to block B7-CTLA-4 interactions to achieve tumor rejection [7]. First, while Ipilimumab can block B7-CTLA-4 interactions when B7-1 and B7-2 are presented in soluble phase, their blocking activity is largely diminished if B7-1 and B7-2 are presented either on the surface of T cells, or immobilized[7] Second, since the in vivo function of CTLA-4 is mediated by transendocytosis of B7-1 and B7-2, we evaluated whether blocking transendocytosis was necessary to achieve the therapeutic effect of anti-CTLA-4; while the Ipilimumab Fab was much less effective in blocking transendocytosis than another Fab anti-CTLA-4 antibody (L3D10), it was comparable to L3D10 in enabling tumor rejection in multiple cancer models in human *CTLA4* gene knock in mice [7] Third, antibody variants devoid of almost all blocking activity showed no reduction in anti-tumor activity, relative to its parent L3D10 antibody.[7] Fourth, by using mice in which half of the CTLA-4 molecules could not bind to anti-human CTLA-4 antibodies-- and thus, no more than 50% of blockade was achieved by anti-human CTLA-4 antibodies -- we observed a strong anti-human CTLA-4 mAb-mediated therapeutic effect in mouse tumor models, regardless of whether the antibodies harbored blocking activity or not [7]. These data, when considered together with data that the ability to engage FcR is required to achieve anti-CTLA-4 antibody-mediated tumor rejection in the mouse, suggest that blocking B7-CTLA-4 interactions may be neither necessary nor sufficient for tumor rejection.; by contrast, we posit that depleting of Tregs in the tumor is responsible tumor rejection, at least for mouse models, as illustrated in Fig. 1.

Differentiating irAE and the Cancer Therapeutic Effect of anti-CTLA-4 antibodies

Given the strong irAE associated with anti-CTLA-4 antibodies in humans [1, 17], an important question for developing next generation anti-CTLA-4 antibodies is whether the therapeutic effect can be disassociated from irAE. The traditional view is that the immunotherapeutic effect can be achieved through antagonist activity of an anti-CTLA-4 antibody, i.e. blocking a checkpoint that can prevent autoimmunity or certain immune responses in cancer [3]. If this is the case, it might be exceedingly difficult to achieve cancer immunity without irAE. However, since an anti-tumor effect can be achieved via selective intratumor Treg depletion, it may be theoretically possible to achieve an anti-tumor effect without irAE. Future studies are warranted to better elucidate this point.

Nevertheless, such antibodies can be identified through functional screenings, as observed in humanized CTLA-4 knockin mice [18]. By comparing mice with one or two alleles of the human *CTLA4* gene, an IrAE can be induced, if, and only if, 100% of the CTLA-4 molecules can be targeted; by contrast, the anti-cancer therapeutic effect requires engagement of no more than 50% of CTLA-4 molecules in mice [18]. The requirement for engaging all CTLA-4 molecules is consistent with the notion that irAE depends on the antagonist activity of the antibody, while the tumor therapeutic effect seems to depend on the agonist activity. We argue that a safe and effective antibody constitutes a Treg-depleting agonist that does not antagonize the endogenous CTLA-4 function, while an effective but irAE-prone antibody bears both agonist and antagonist activities. By extension, those antibodies that bear antagonist but no agonist activity (by having a non-depleting isotype) would be irAE prone without much therapeutic effect. Further studies are evidently needed to understand the molecular and cellular mechanisms by which anti-CTLA-4 mAbs may cause irAE. Such data would allow more rationale design of safe and effective anti-CTLA-4 antibodies for human cancer therapy. ?

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Glossaries

Fab	antigen-binding fragment of immunoglobulin
Fc	crystallizable fragment of immunoglobulin
FcR	receptor for crystallizable fragment of immunoglobulin
Tregs	regulatory T cells

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How do anti-CTLA-4 mAbs cause tumor rejection?

Fig. 1. Two Mechanisms of Action of Anti-CTLA-4 Antibodies in Cancer Immunotherapy.

The traditional view of anti-CTLA-4 antibody immunotherapy (a) considers the mechanism of checkpoint blockade as the driving force for tumor rejection, while the new model (b) considers regulatory T cell (Treg) depletion as the driving force. ADCC: Antibody-dependent cell-mediated cytotoxicity; ADCP: antibody-dependent cell-mediated phagocytosis; APC: antigen-presenting cells.