Science Bulletin 64 (2019) 1388–1389

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Science Bulletin
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Research Highlight

Degradation of CTLA-4 balances toxicity and efficacy

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The notorious side effects of anti-CTLA-4 limit its use in the clinic, but what causes the severe side effects remains largely unclear. Zhang et al. [1] elucidated antibody-induced CTLA-4 lysosomal degradation as a new mechanism accounting for the severe immunotherapy-related adverse effects and limited efficacy of anti-CTLA-4 therapy.

CTLA-4 expresses on regulatory T cells (Tregs) and activated CD8 and CD4 effector cells. Upon high affinity binding to its ligand CD80 and CD86 on antigen-presenting cells (APCs), CTLA-4 limits the further activation of effector cells and plays an essential role in maintaining the suppressive function of Tregs. Therefore, the anti-CTLA-4 antibodies can remove the suppressive function of Tregs and release the cytotoxicity function of effector cells. The anti-CTLA-4 (Ipilimumab) immunotherapy is the first immune checkpoint blockade (ICB) that was approved by the FDA for treating metastatic melanomas [2]. Despite the prolonged survival of patients, anti-CTLA-4 antibody treatment can cause severe immunotherapy-related adverse effects (irAEs), which significantly limits its clinical benefits. Therefore, more studies turn to combine low-dose anti-CTLA-4 immunotherapy with the anti-PD-1 blockade. Even with a reduced dose of anti-CTLA-4 antibodies, the combination therapy with anti-PD-1 treatment can cause severe irAEs in many patients [3]. It is imperative to understand the causes of side effects in anti-CTLA-4 immunotherapy and developing new approaches accordingly.

In a recent paper published in *Cell Research*, Zhang et al. [1] demonstrate that antibody-induced CTLA-4 lysosomal degradation leads to irAEs and compromised efficacy. By comparing four different clones of anti-human CTLA-4 antibodies, the authors observed that irAE-prone Ipilimumab and TremeIgG1 down-regulated the CTLA-4 proteins but not for the non-irAE-prone antibodies (HL12 or HL32). Next, they found that irAE-prone antibodies-bound CTLA-4 were internalized and degraded in the lysosome. In contract, non-irAE-prone antibodies released CTLA-4 at low pH condition in the late endosome and the latter underwent lipopolysaccharide-responsive and beige-like anchor (LRBA)-dependent recycling to cell membrane (Fig. 1). On the one hand, they showed that inhibiting the endocytic recycling process that blocked CTLA-4 recycling led to the diminution of CTLA-4 cell surface expression by non-irAE-prone anti-CTLA-4 antibodies. On the other hand, increasing the pH sensitivity of irAE-prone anti-CTLA-4 antibodies dramatically increased CTLA-4 recurrence on cell surface. At last, the paper shows reduced low-pH binding affinity improves the efficacy of irAE-prone anti-CTLA-4 antibodies.

The mechanisms of how anti-CTLA-4 works in clinics are still under debate and most of the studies focused on how anti-CTLA-4 antibodies affected the function of effector and Tregs. Zhang et al. [1] proved that clinically used anti-CTLA4 caused CTLA-4 degradation and irAE in human-CTLA-4 transgenic mice, but modulated pH-sensitive anti-CTLA4 antibodies reduced CTLA-4 degradation and relieved irAE. This result is consistent with previous studies showing CTLA-4-deficient mice developed severe autoimmune diseases. The recycling of CTLA-4 between the cytoplasm and the cell surface was observed a long time ago [4] and its interactions with the endosomal protein LRBA are important for CTLA-4 recycling [5]. The further experiments demonstrate that CTLA-4 recycling is essential for reduced irAE: (1) irAE-prone antibodies are rapidly colocalized with lysosomes; (2) Very few recycling CTLA-4 are detected after irAE-prone antibodies treatment; (3) The inhibition of lysosomal degradation prevents the downregulation of CTLA-4 by irAE-prone antibodies; (4) The difference in CTLA-4 recycling is caused by differential pH sensitivity of anti-CTLA-4 antibodies. Low-pH release of CTLA-4 by non-irAE-prone antibodies will lead to more CTLA-4 recycling in an LRBA-dependent way. There are also other studies trying to reduce the side effect of anti-CTLA-4 treatment in different ways. Fransen et al. [6] showed that local delivery of anti-CTLA-4 antibodies increased the CD8 T cell function and reduced toxicity. A recent study by Fong's group [7] utilizing the bispecific antibodies targeting tumor-associated antigens and CTLA-4 showed a reduced irAE by sparing Tregs in peripheral tissues. These two studies suggest that the side effect of anti-CTLA-4 is caused by depletion of Tregs in the peripheral tissues. TNF blockade can also alleviate the irAEs induced by dual CTLA-4 and PD-1 immunotherapy [8]. Compared to these studies, the approach by Zhang et al. can alternatively reduce the irAEs induced by anti-CTLA-4 treatment.

Despite the reduction of Tregs is not detected in the clinic, some studies suggest that the therapeutic function of anti-CTLA-4 depends on the depletion of intratumoral Tregs [9]. Increasing the ADCC effect of anti-CTLA-4 will increase the efficacy of anti-CTLA-4 treatment [10]. Zhang et al. [1] showed that increasing the recycling of CTLA-4 by pH-sensitive antibodies lead to more Treg-cell depletion and improved therapeutic effects. First,

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https://doi.org/10.1016/j.scib.2019.07.028

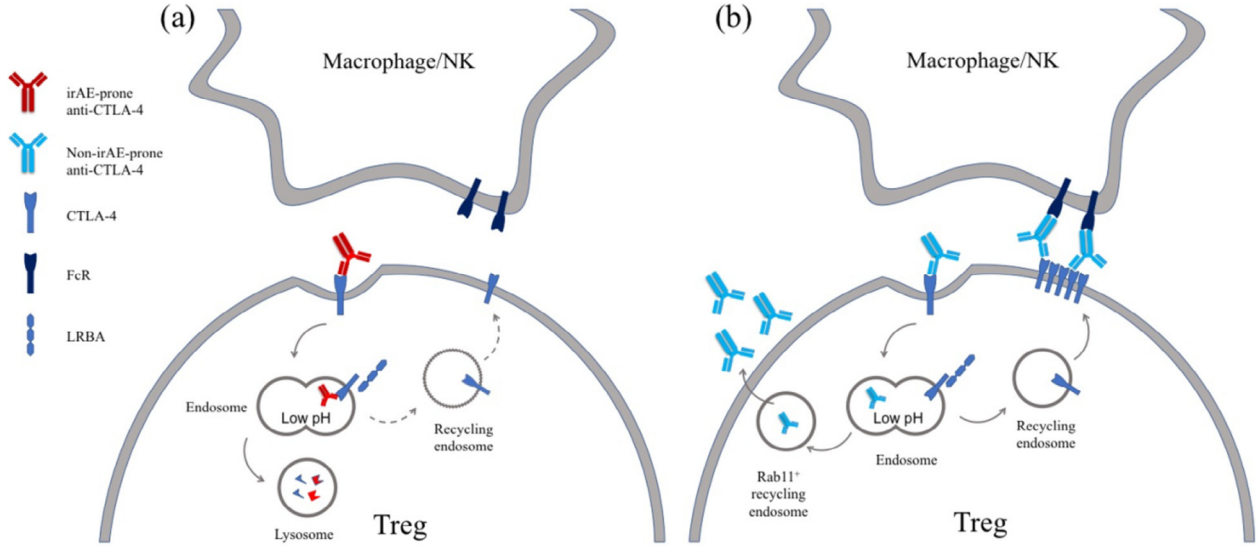

Fig. 1. (Color online) Antibody-induced CTLA-4 lysosomal degradation regulates toxicity and efficacy. (a) Immunotherapy-related adverse effect (irAE)-prone anti-CTLA-4 antibodies can bind CTLA-4 at low pH in the endosome, which inhibits LRBA-mediated CTLA-4 recycling and increases the degradation of CTLA-4 and antibodies. Reduced CTLA-4 expression on the surface is related to anti-CTLA-4-induced irAEs. (b) Non-irAE-prone anti-CTLA-4 antibodies release CTLA-4 under the low pH condition in the endosome, which allows LRBA-mediated recycling of CTLA-4 and anti-CTLA-4 recycling via Rab11$^+$ recycling endosome. The increased expression of CTLA-4 results to enhanced ADDC (antibody-dependent cellular cytotoxicity) or ADCP (antibody-dependent cellular phagocytosis) with low toxicity.

pH-sensitive antibodies increased the recycling of CTLA-4. Therefore, there will be more CTLA-4 on the surface for better ADCC (antibody-dependent cellular cytotoxicity). Second, pH-sensitive antibodies can recycle to the outside of cells with less degradation, so there will be more antibodies in tumor microenvironments. The location is important for the understanding of efficacy and toxicity of anti-CTLA-4 treatment, but this is not studied in this paper. Therefore, Tregs in the tumor tissues with higher expression of CTLA-4 can be preferentially depleted than those in peripheral tissues. This will restore the effector function of CD8 and CD4 T cells. Importantly, irAE-prone anti-CTLA antibodies can induce the degradation of CTLA-4, which is important for the suppressive function of Tregs, and losing suppressive function of Tregs in the peripheral tissues accounts for the severe irAEs of anti-CTLA-4 treatment. With better understanding of the mechanisms underlying how anti-CTLA-4 antibodies work and how irAEs are induced, more effective and less toxic anti-CTLA-4 treatment can thus be developed.

Conflict of interest

The authors declare that they have no conflict of interest.

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Yang-Xin Fu, M.D., Ph.D., Professor of Pathology, studies immunotherapy and immunopathology in cancer and infectious diseases. His current research interests include investigating and understanding the mechanisms underlying IR-induced extrinsic resistance as well as testing newly developed personalized immunotherapies to overcome these resistance mechanisms for improved and long-lasting tumor control.