Braking Bad: Blockade of Inhibitory Pathways Improves Interleukin-15 Therapy

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Blockade of the CTLA-4 and PD-1 inhibitory pathways in T cells via the administration of neutralizing antibodies at the time of interleukin (IL)-15 therapy markedly enhanced the survival of tumor-bearing mice as compared with those receiving IL-15 alone or IL-15 in combination with just one of the antibodies. Clin Cancer Res; 16(24): 5917–9. ©2010 AACR.

In this issue of Clinical Cancer Research, Yu and colleagues have investigated the antitumor effects of interleukin (IL)-15 in a murine model of metastatic cancer and have shown that the removal of key intercellular inhibitory checkpoints leads to a significant improvement in efficacy (1). The eradication of advanced cancers via the administration of T-cell stimulatory cytokines has been a goal of translational immunologists since the discovery of IL-2 in the 1980s. In contrast to the successes obtained in murine models in which cytokine administration can lead to the complete eradication of bulky disease, the results of human clinical trials have been more modest: High-dose IL-2 therapy leads to significant tumor shrinkage in about 15% of patients with stage IV melanoma or renal cell cancer (2).

Investigators now know that the immune system is populated with inhibitory mechanisms that act to brake the specific immune response. These negative feedback systems include deactivators of cytokine signal transduction (e.g., suppressor of cytokine signaling proteins), cell-to-cell interactions involving the binding of ligands on antigen-presenting cells to T-cell inhibitory receptors (PD-1 and CTLA-4), and distinct cellular compartments that have potent immune-suppressive properties (regulatory T cells and myeloid-derived suppressor cells; refs. 3–6). Importantly, neutralization of these inhibitory pathways can enhance the effectiveness of exogenously administered cytokines. Administration of a neutralizing anti-CTLA4 antibody to patients with advanced melanoma leads to significant reductions in tumor volume in a small but reproducible subset of patients. Interestingly, one unusual side-effect of this class of drugs is the induction of autoimmune reactions such as colitis, dermatitis, and hypophysitis, which is an indirect testimony to the potency and importance of this pathway in the prevention of unwanted immune reactions (7).

IL-15 is a member of the four alpha-helix bundle family of cytokines and is unique among so-called “gamma chain” cytokines, in that it is presented on the surface of IL-15–producing cells tightly bound to the IL-15R alpha chain. This alpha chain/IL-15 complex interacts with the IL-2/15beta chain and the common receptor gamma chain of T cells to form a high-affinity heterotrimeric receptor that mediates the activation of downstream effector pathways such as cellular cytotoxicity and IFN-gamma secretion. IL-15 is critically important for the homeostasis of memory CD8+ T-cell populations and is responsible for the recovery of naive T cells under lymphopenic conditions. IL-15 is also an important survival factor for human natural killer cells. And, unlike IL-2, IL-15 does not seem to stimulate the outgrowth of regulatory T cells. Thus, IL-15 exhibits a number of properties that make it a desirable cytokine for exogenous administration to patients with advanced cancers (8).

Yu and colleagues examined the antitumor effects of IL-15 treatments in mice that had received tail vein injections of 2 × 105 CT26 adenocarcinoma tumor cells (1). In the absence of therapy, these mice developed extensive lung metastases and died within about 21 days. In contrast, the survival of mice treated with murine IL-15 (5 µg intraperitoneally five days a week for 3 weeks) was significantly prolonged, and this effect was dependent on the actions of CD8+ T cells, which exhibited increased lytic activity and IFN-gamma production in response to CT26 cells. However, a further analysis of the CD8+ T-cell population revealed that the effects of IL-15 therapy were not all positive; there was also a significant increase in the expression of the PD-1 inhibitory receptor and secretion of IL-10 (a potent immune-suppressive cytokine) in the CD8+CD44high memory T cells of IL-15–treated mice. The administration of a blocking antibody to CTLA-4 or a neutralizing antibody specific for a ligand of PD-1 (PD-L1) each had modest effects on survival in the absence of any other manipulations. Importantly, coadministration of the anti-CTLA4 antibody and the anti–PD-L1 antibody at the time of IL-15 therapy effectively reversed the expression of PD-1 and IL-10 and markedly enhanced the survival of tumor-bearing mice as compared with those receiving IL-15 alone or IL-15 in combination with just one of the
antibodies (see Fig. 1). The effect of combined inhibition of CTLA-4 and PD-1 in the context of IL-15 therapy was robust and statistically significant; however, complete cures were not obtained. Although in depth analyses of organs that might be the target of autoimmune reactions were not done, the regimen was well tolerated.

CTLA-4 and PD-1 both block T-cell activation via the inhibition of Akt activation, but they accomplish this via different means. Engagement of PD-1 inhibits phosphoinositide 3-kinase (PI3K) activity, which is upstream of Akt, whereas signaling by CTLA-4 works through the phosphatase PP2A to inhibit Akt phosphorylation. It is likely that dual blockade of CTLA-4 and PD-1 results in more complete inhibition of Akt, but the superiority of this strategy may also depend on parallel signaling pathways that are modulated by the activity of PI3K and PP2A. The utility of this strategy is underscored by the recent work of Curran and colleagues who employed the combination of CTLA-4 and PD-1 blockade in the context of a whole-cell antitumor vaccine and found that this combined approach resulted in an increase in tumor-infiltrating T cells and a reduction in regulatory T cells within the tumor (9).

Several key observations can be made about this study. First, it is clear that the neutralization of ligand-receptor interactions within the CTLA4 family of inhibitory molecules markedly enhances the effectiveness of IL-15 therapy. This finding is important and may lead clinicians to reexamine the utility of other T-cell stimulatory cytokines that have not entered into routine clinical use because of toxicities that occur when these cytokines are administered at high doses (e.g., IL-2). Also, the further development of cytokines that are currently under clinical investigation and have shown preliminary promise (e.g., IL-7, IL-21) may be benefited by attention to the potential advantages of administering the cytokine in conjunction with CTLA-4/PD-1 inhibition (10, 11). Secondly, there seems to be a distinct advantage to the simultaneous inhibition of multiple inhibitory pathways. This finding raises the question whether the inhibition of additional pathways might lead to increased antitumor activity for IL-15 and other cytokines. For example, B- and T-lymphocyte attenuator (BTLA) is a novel inhibitory receptor with structural and functional similarities to CTLA-4 and PD-1. Triggering of BTLA leads to decreased antimicrobial and autoimmune T-cell responses in mice, and downregulation of this receptor in humans via the administration of TLR agonists promotes the CD8⁺ T-cell response to a defined vaccine (12). It is conceivable that future strategies could involve the
simultaneous inhibition of multiple inhibitory pathways regulating T-cell activation. The study by Curran and colleagues suggests that the incorporation of strategies to eliminate immune suppressor cells may also improve the T-cell response to cytokine treatments (9). The factors that promote the growth and survival of regulatory T cells and myeloid-derived suppressor cells and the factors that mediate their immunosuppressive effects are also becoming better defined, and it is conceivable that the future of cancer immunotherapy will involve antibody neutralization strategies aimed at these inhibitory pathways.

Recent work suggests that IL-15 may be quite effective in a variety of clinical situations. The present study also suggests that targeted neutralization of negative feedback pathways can greatly augment the efficacy of this cytokine.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


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