

CTLA-4: From conflict to clinic

CTLA-4 was first identified in 1991 as a second receptor for the T cell costimulation ligand B7. Uncertainties about its biological function plagued the early years after its discovery until 1995, when it was confirmed to be an inhibitor of T cell responses. CTLA-4 has since scored in the clinic as a target for antitumor therapy and as a soluble inhibitor of autoimmunity.

By the late 1980s, it was clear that naive T cells require two signals through two different receptors to spark to life. Tickling the T cell receptors (TCRs) alone with antigenic ligands makes the cells anergic. But a second signal from the B7 ligand on antigen-presenting cells (APCs) to the CD28 coreceptor goads T cells into action (1). As researchers pursued the mechanism of B7-CD28-mediated T cell costimulation, another T cell surface molecule was generating interest due to its homology with CD28.

Another T cell switch...

In 1987, researchers hunting for cytotoxic cell surface molecules isolated a cDNA from activated CD8⁺ T cells and called it cytotoxic T cell antigen (CTLA)-4 (2). Genetic studies provided a clue that connected CTLA-4 to T cell costimulation: CTLA-4 and CD28 both mapped to the same chromosomal neighborhood and shared a high degree of sequence similarity (3).

This information caught the attention of Jeffrey Ledbetter and Peter Linsley at the Bristol-Myers Squibb Research Institute (Seattle, WA). At the time, their group was studying B7-CD28 interactions using a soluble version of CD28 that lacked trans-membrane and intracellular domains. The soluble CD28 protein worked just as well as the cell-attached version in binding B7 (4). So when CTLA-4 appeared on the radar, Linsley constructed a soluble CTLA-4 protein. The team hedged their bets on the similarities between CTLA-4 and CD28 and tested whether CTLA-4 also bound B7.

Soluble CTLA-4 turned out to bind B7 with 20-fold higher avidity than the

soluble CD28 protein, establishing CTLA-4 as a second receptor for B7. The group published these results in *The Journal of Experimental Medicine* in 1991 (5). It now seemed that CTLA-4 was a third switch in what was previously thought to be a two-switch circuit for T cell activation. But whether it promoted or jammed the circuit was a contentious issue for several more years.

...but on or off?

The debate arose because in vitro assays of costimulation-dependent T cell proliferation offered multiple interpretations. T cells normally proliferate in vitro when cultured with B7-expressing APCs. But when soluble CTLA-4 was added to the mix, this proliferation was strongly inhibited (5).

One interpretation of this finding was that the soluble CTLA-4 blocked the binding of B7 to the CTLA-4 on the T cells. In this model, the T cell CTLA-4 was needed for proliferation, along with CD28. This conclusion was supported when the group showed that antibodies to CTLA-4 enhanced CD28-stimulated proliferation (6).

A second interpretation, however, was that soluble CTLA-4 might block proliferation by gumming up B7's interaction with CD28. And the CTLA-4 antibody might enhance proliferation not because it stimulates CTLA-4's proliferative power, but because it blocks CTLA-4's negative signal.

This alternative role for CTLA-4 was supported by the work of James Allison and his team at the University of California (Berkeley, CA), which was also published in the *JEM* (7). This group studied cross-talk among TCR, CD28, and CTLA-4 by cross-linking these receptors. T cells proliferated when the TCR was linked to CD28, but not with CTLA-4.



Jeffrey Ledbetter (left) and James Allison

T cell proliferation was greatly reduced when all three receptors were cross-linked simultaneously, suggesting that CTLA-4 inhibits CD28 costimulation.

The generation of CTLA-4 knockout mice finally put the conflict to rest. These animals develop a fatal T cell proliferative disorder, as their T cells lack the brakes to hold them in check (8). CTLA-4 was thus established as a negative regulator of T cell function and proliferation.

CTLA-4 in the clinic

One benefit of enhancing T cell responses via CTLA-4 blockade is the strengthening of antitumor immunity. Allison's team found that tumor-transplanted mice injected with antibodies that block CTLA-4 activity rejected several different types of tumors and had long-lasting antitumor immunity (9). Human anti-CTLA-4 mAbs are now in phase III clinical trials against melanoma and renal carcinomas. CTLA-4 has also been called into service to turn off dangerous immune responses. The T cell-inhibiting soluble CTLA-4 originally defined by Linsley and Ledbetter is now used to treat autoimmune diseases such as rheumatoid arthritis.

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