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Pillars of Immunology
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CTLA-4: Not All Costimulation Is Stimulatory

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To activate a T cell, it was widely accepted, even in the 1960s, that there had to be a "signal one," namely, receptor recognition of a foreign Ag. That fact did not make it less exciting when, in the early 1980s, Abs to the TCR were generated and the genes that encode it identified (1). As a result, we learned about the TCR structure and the signal transduction pathways that turned "signal one" into action (2).

The story became more interesting when we realized that to activate a naive T cell, "signal one" had to be joined by "signal two." This idea meshed well with theories put forward by Bretscher and Cohn (3), as well as previous work by Lafferty (4), on the need for "passenger leukocytes" to activate graft rejection. It was given additional substance with the demonstration in vitro that engagement of the TCR alone not only failed to induce effective T cell activation but also actually induced T cell anergy (5). Before long, we had learned that "signal two" could be provided by the engagement of CD28 on the T cell by its ligands on APCs, B7-1 (CD80), and/or B7-2 (CD86), thereby producing costimulation.

The molecule we now call CD28 was first defined in the early 1980s by a mAb called 9.3 that bound to almost all resting human $CD4^+$ cells and ~50% of $CD8^+$ cells (6). The mAb 9.3 activated numerous signaling pathways and, while having little or no effect on resting T cells, cooperated with TCR stimulation to induce optimal cytokine production, proliferation, and cell survival (7). It is noteworthy that although many immunologists today lament the paucity of effort devoted to human, compared with mouse, immunology, the first well-characterized lymphocyte cell surface molecules, including CD28, were identified in humans, using mAbs.

By the late 1980s, it was becoming clear that a family of costimulatory molecules did, in fact, exist. In 1987, CTLA-4 was described as the fourth in a series of gene products identified in a subtractive cDNA library produced from activated CTLs (hence CTL activation gene number four, or CTLA-4) (8). In a key observation, CTLA-4 soon was found to have significant sequence homology to CD28 and to bind to CD80 and CD86. In addition, Ab binding to CTLA-4 enhanced T cell activation, as had Abs directed at CD28 (9). Thus, in the early 1990s, it was concluded that CTLA-4 functioned like CD28; our understanding of T cell activation was that all signals were positive; everything was a go. Yet, some features of CTLA-4 distinguished it from CD28. CTLA-4, unlike CD28, was not expressed on resting T cells and appeared only after T cell activation. In addition, CTLA-4 (also called CD152) had significantly higher binding affinity for the B7 ligands than did CD28, suggesting that perhaps the story was not so simple.

Then, in the early 1990s, came the publication of the two papers selected as this issue's *Pillars of Immunology* (10, 11). In essence, both papers started with the recognition that an experimental manipulation that appeared to be activating an activator (in this case, anti–CTLA-4 Abs enhancing T cell activation) would give the same outcome if the manipulation was actually inhibiting an inhibitor.

These two critical papers showed that although anti–CTLA-4 Abs enhanced T cell activation under some circumstances, this enhancement probably was not because the Abs were stimulatory, for four reasons: 1) the combination of anti-CD3 plus anti–CTLA-4 Abs was not sufficient to initiate T cell activation, in contrast to anti-CD3 plus anti-CD28; 2) Fab Abs of anti–CTLA-4 enhanced T cell activation, whereas Fab anti-CD28 Abs were inhibitory; 3) anti–CTLA-4 Abs inhibited activation under conditions of maximal crosslinking; and 4) blockade of B7 molecules, while simultaneously stimulating CD28 with anti-CD28 Abs, led to enhanced activation. Taken together, this powerful evidence pointed to the fact that CTLA-4 was a T cell-inhibiting molecule, not a stimulating one. Suddenly, the field of costimulation had become a lot more complicated —and a lot more interesting. T cell activation by "signal one" was modulated not only by positive costimulatory signals but by negative regulating molecules as well. Just how important these negative signals are was revealed when CTLA-4 knockout mice were found to die at an early age owing to massive lymphoproliferation involving visceral organs (12, 13).

Since the publication of these two *Pillars of Immunology* articles, the list of positive and negative costimulatory molecules has grown substantially, with the addition of ICOS, PD-1, and others expressed on T cells, along with their ligands on APCs (14). In addition, we have learned that many soluble factors can modulate T cell responses as well. Today, we appreciate that the regulation of T cell activation is finely tuned by an elaborate interaction of molecules that function at different times and sites. However, we could not begin to unravel this complexity until we understood that we were looking for negative as well as positive signaling molecules, making publication of these manuscripts a turning point in the story.

The discovery of CTLA-4 and the recognition that it provided a negative T cell signal resulted from outstanding basic research; however, an appealing part of the story is that this basic research has led directly to important clinical advances. CTLA4Ig (abatacept) has been approved by the U.S. Food and Drug Administration for treatment of rheumatoid arthritis, and a Food and Drug Administration advisory panel has

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recommended approval for use in transplantation of a mutated form of CTLA4Ig (belatacept) that has higher ligand-binding affinity, especially for CD86. Furthermore, an anti–CTLA-4 Ab (ipilimumab), which blocks the inhibitory effect of CTLA-4 on the T cell response, including the antitumor T cell response, has been approved for treatment of advanced malignant melanoma. Thus, the story of CTLA-4 is one that taught us an important immunologic principle about T cells and that also went from the bench to the bedside.

In retrospect, the complexity of T cell costimulation seems obvious. What biological system can we think of that uses activating pathways and does not also have counterbalancing inactivating pathways? Lots of ideas are obvious in retrospect, however. These two *Pillars of Immunology* articles considered a set of observations from an entirely opposite point of view and in the process guided us toward an understanding that not all T cell costimulation is stimulatory.

Disclosures

H.A. has family members with a financial interest in Bristol-Meyers Squibb. L.A.T. has family members with a financial interest in Novartis.

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