

Distinct roles for CD28 and Cytotoxic T Lymphocyte-associated Molecule-4 Receptors during T Cell Activation?

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Cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) is a lymphocyte cell surface receptor originally discovered in a search for molecules having a role in T cell cytotoxicity (1). This molecule is a member of the immunoglobulin superfamily and is homologous to another T cell surface receptor, CD28. Both CD28 and CTLA-4 bind the same counter-receptors, members of the B7 family on APCs. While the role of CD28 during T cell responses to antigen has been well studied, much less is known about the role of CTLA-4. Recent studies on the function of this molecule have been controversial and have yielded seemingly conflicting results. In this issue, a new study (2) provides further evidence on the function of this molecule and lends support to growing evidence that CTLA-4 is a receptor having unique function during an immune response. What follows in this article is an account of the development of this story, an analysis of the significance of the new data, and a discussion of potential fruitful areas of future research on CTLA-4.

Role of B7 Molecules in T Cell Costimulation. The interactions of T lymphocytes with APC are key to the generation of an immune response to foreign pathogens, transplanted organs, or to self tissue during autoimmune disease. The specificity of these T cell-APC interactions is provided by the recognition of antigenic peptide-MHC complexes by clonotypic TCR. However, TCR engagement alone generally does not lead to full T cell activation, but may instead lead to T cell clonal anergy. A successful immune response requires additional interactions between the T cell and the APC. These costimulatory interactions thus determine the outcome of TCR engagement, i.e., whether this engagement activates or inactivates subsequent immune responses (3). While the molecular nature of these costimulatory interactions is not fully understood, it is now clear that a key T cell costimulatory signal is provided by interaction of CD28 receptors on T cells with B7 counter-receptors on APC (4). Engagement of the CD28 receptor by B7 molecules triggers a signaling pathway that regulates T cell cytokine production, particularly IL-2 (4). Two B7 molecules are known, B7-1 (CD80) and B70/B7-2 (CD86), each of which binds CD28 with similar avidity and elicits similar functional effects (5).

CTLA-4. CTLA-4 is a second high avidity receptor for B7 molecules that was cloned from a subtracted cytolytic T cell cDNA library (1, 6). CTLA-4 transcripts were detected in activated lymphocytes and were coinduced with T cell cytotoxicity. Human and mouse genes encoding CTLA-4 map to the same chromosomal band as CD28 (7, 8), and human

CTLA-4 and CD28 have been linked at the molecular level on a yeast artificial chromosome clone (9). Recombinant soluble CTLA-4 binds CD80 and CD86 with higher avidity than recombinant soluble CD28 (5, 10).

Although originally identified as a cytolytic T cell-associated molecule, CTLA-4 transcripts have been detected in both CD4⁺ and CD8⁺ T cell clones (11). Cell surface expression of CTLA-4 on activated T cells has been detected using specific mAbs. In human T cells (12), CTLA-4 is expressed equally on CD4⁺ and CD8⁺ T subsets of activated T cells, whereas with murine cells, CTLA-4 expression is higher on the CD8⁺ subset (13). Expression of CTLA-4 is highly activation dependent; CTLA-4 is not detected on resting cells but is induced during T cell activation (1, 11-13). Expression is regulated in part by levels of mRNA (1, 11). With activated human T cells, maximal CTLA-4 protein expression was ~2-3% of CD28 (12). CTLA-4 is therefore a high avidity, low abundance receptor for B7 molecules.

Functions of CTLA-4 during T Cell Activation. Relatively little is known of the role of CTLA-4 during T cell activation, and the few experiments reported are largely contradictory. Complete conservation was noted in the amino acid sequences of the cytoplasmic tails of murine and human homologues of CTLA-4 (14). Since the cytoplasmic tail of CTLA-4 presumably mediates signal transduction, this sequence conservation suggested that CTLA-4 has an important conserved signaling function. The cytoplasmic tails of CTLA-4 and CD28 show more limited sequence homology (7), so it was therefore difficult to predict from sequence comparisons alone whether CTLA-4 would have similar or different functions than CD28.

Initial experiments (12) showed that combinations of CTLA-4 mAb and anti-CD28 mAb or Fab fragments were synergistic in blocking adhesion of activated CD4⁺ lymphocytes to CD80-transfected CHO cells, and in blocking T cell proliferation during a mixed lymphocyte reaction (MLR). Anti-CTLA-4 mAb had weak costimulatory activity together with anti-TCR mAb on previously primed CD4⁺ T cells, but the effects were less than those observed with anti-CD28 mAb plus anti-TCR mAb. Combinations of suboptimal amounts of anti-TCR mAb, anti-CD28 mAb plus anti-CTLA-4 mAb were synergistic in their costimulatory ability (12). Another study from this group (15) extended the original findings and showed that the cooperative costimulatory effects of anti-CTLA-4 and anti-CD28 mAbs were greatest at low concentrations of anti-CD28 mAb (and, hence, low occupancy

of CD28 receptors). Taken together, these observations led to the proposal that CD28 and CTLA-4 cooperatively regulate T cell activation and costimulation by B7 molecules.

A more recent study by Walunas et al. (13) suggested that CTLA-4 can also serve as a negative regulator of T cell activation. These investigators showed that in contrast to previous studies with human lymphocytes, anti-CTLA-4 mAb increased T cell proliferation in murine MLR. Similar results were obtained with Fab fragments of anti-CTLA-4 mAb. These results were interpreted to mean that anti-CTLA-4 mAb elicited its stimulatory effects by blocking interactions of CTLA-4 with its natural ligand, an interaction that was inhibitory for T cell proliferation in the MLR. Additional support for this proposal came from the observation that anti-CTLA-4 mAb inhibited proliferation of T cells costimulated with anti-CD3 and anti-CD28 mAbs. These studies led to the proposal that CTLA-4 interaction with its natural ligand downregulates an immune response.

Another recently published study (16) also suggested an antagonistic function for CTLA-4. These authors showed that anti-CTLA-4 mAbs triggered antigen-specific apoptosis in previously activated human T cells. With naive T cells, however, anti-CTLA-4 mAbs provided agonistic effects to combinations of anti-CD3 and anti-CD28 mAbs. Thus, the effects of anti-CTLA-4 mAbs were determined by the activation state of the cells studied.

Thus, these initial studies have led to two different and distinct models for the role of CTLA-4 in T cell activation (Fig. 1). In one model, CD28 and CTLA-4 function cooperatively to upregulate T cell activation; in the other model, CTLA-4 antagonizes CD28 and downregulates T cell activation.

The new study by Krummel and Allison (2) provides new data on the function of CTLA-4. These authors show that when used together with anti-CD3 mAb immobilized on

plastic wells, anti-CTLA-4 and anti-CD28 mAbs were synergistic in their ability to costimulate the proliferation of murine T cells. These data were interpreted to mean that anti-CTLA-4 mAb blocked an inhibitory effect on T cell proliferation caused by interaction between endogenous B7 molecules on T cells with CTLA-4. Other experiments showed that cross-linking of anti-CD3 plus anti-CD28 mAbs had a powerful costimulatory effect on T cell proliferation, cross-linking of anti-CTLA-4 mAb together with the other antibodies inhibited T cell proliferation. When the TCR, CD28, and CTLA-4 mAbs were immobilized on plastic beads, anti-CTLA-4 mAbs inhibited the proliferative effects of anti-CD28 plus anti-CD3 mAbs. Increasing amounts of anti-CTLA-4 mAbs progressively inhibited the costimulatory effects of anti-CD28 mAbs.

Thus, anti-CTLA-4 mAbs can either stimulate or inhibit T cell activation, depending on experimental conditions. The study by Krummel and Allison demonstrates several factors that are critical for determining the direction of the effects of anti-CTLA-4 mAbs. These include the endogenous expression of B7 by T cells. A mixture of mAbs to B7-1 and B7-2 showed similar stimulatory effects as anti-CTLA-4 mAb when the TCR signal was provided by mAb immobilized on plastic wells. Since B7-2 was expressed at low (nonstimulatory) levels on the T cells used in these experiments, T cell-T cell interactions mediated by interaction of B7-2 and CTLA-4 may have been inhibitory for T cell proliferation. It is unclear how endogenous expression of B7 affects the inhibitory effects of anti-CTLA-4 mAbs seen under other conditions. Also, the degree of co-cross-linking of CD3, with CD28, and/or CTLA-4 was important for determining the extent of proliferation. Taken together, the data suggest that the outcome of the T cell antigen receptor engagement is determined by integration of signals provided by CD28 and CTLA-4.

What do these findings tell us about the role of CTLA-4 in T cell activation? Perhaps most simply, they suggest that stimulation of CD28 and CTLA-4 TCRs may have different effects on T cell activation. Does this mean that CTLA-4 and CD28 have intrinsically opposite effects during T cell activation? Not necessarily. Experience in other signaling systems has taught us that it is quite common for receptor triggering to be context dependent, i.e., opposite effects are elicited under different experimental conditions. For example, it has long been known that cytokines and their receptors are multifunctional (17); many cytokines can stimulate proliferation under certain conditions and inhibit under others. The reasons for these different effects are not fully understood, but may involve differences in receptor occupancy, coupling to the signal transducing receptors, or the presence or absence of other cofactors. We should keep in mind that, generally speaking, a receptor that has only one effect is one that has not been fully studied. Perhaps the main message from the studies of Krummel and Allison is that CTLA-4 has come of age. Thus, we now realize that CTLA-4 is a receptor with its own unique properties and its own mechanisms of integrating with the lymphocyte signal transduction machinery.

Future Directions. As usual, coming of age means that life

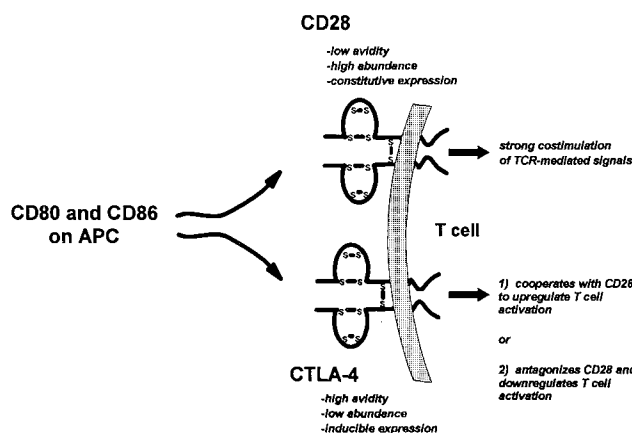


Figure 1. Summary of properties of T cell surface receptors CD28 and CTLA-4: expression, binding, and functional properties. The subunit structure of CD28 and CTLA-4 receptors is indicated schematically; each is homodimeric. The relative positions of inter- and intramolecular disulfide bonds of CD28 and CTLA-4 are depicted. Differences are emphasized in functional properties of CD28 and CTLA-4 as indicated by studies using specific mAbs.

becomes more complicated. The studies of Krummel and Allison also indicate that the process of T cell costimulation is more complicated than previously imagined, and more highly regulated. Since CTLA-4 and CD28 are coexpressed on the same cells and share common ligands, their engagement cannot be considered as independent events. The net result of engagement of CD28 and CTLA-4 receptors by B7 ligands will be determined by (a) the different affinities of CTLA-4 and CD28 for their B7 ligands; (b) the different expression levels of CTLA-4 and CD28; and (c) different effects of signaling through these two receptors (Fig. 1). How these factors are integrated by the lymphocyte signaling machinery is largely unknown at this juncture.

Future experiments will be necessary to more fully elucidate the roles of signals through the CTLA-4 and CD28 receptors. Perhaps all the reported experimental results are correct but their validity is limited to the particular experimental conditions that have been used. Further in vitro studies with human and murine T cells may help clarify the different effects of anti-CTLA-4 mAbs. One area that needs clarification is whether CTLA-4 has different functions in the different lymphocyte subsets used by different groups or in different species. Another area is whether mAbs to different epitopes on CTLA-4 have different effects. Undoubtedly, gene-targeted disruptions of CD28 and CTLA-4 genes will be valuable in future studies. CD28 knockout mice are defective in certain but not other immune responses (18). It will be important to determine the role of CTLA-4 in these mice. One study (19) suggested that CTLA-4 did not function in vitro when lymphocytes from these mice were studied, but more needs to be done, particularly during in vivo immune responses. A report on the properties of CTLA-4 knockout mice is eagerly awaited. Perhaps other transgenic studies will also be useful. If CTLA-4 triggering has a negative effect on T cell activation in vivo, then constitutive expression of CTLA-4 on transgenic T cells should have a negative effect on the immune responses of such animals.

Finally, a more complete understanding of the signal transduction pathways of CTLA-4 and CD28 receptors is needed.

While there is a tendency to attribute some of the effects of anti-CTLA-4 mAbs as evidence for a "negative signal" transmitted by this receptor (2, 13), it is unclear what this term means. Despite the importance of CD28 receptor triggering during T cell activation, relatively little is known of its signal transduction pathway(s) and even less is known of pathway(s) used by CTLA-4. It is perhaps typical of our state of knowledge about CTLA-4 that the two published studies (20, 21) on its signaling have given conflicting results. Both of these studies examined whether CTLA-4 triggering stimulated association of PI-3 kinase with a Y-X-X-M motif in its cytoplasmic tail, as has been demonstrated with a similar motif in the CD28 cytoplasmic tail (reviewed in reference 22). Stein et al. (20) found that CD8-CTLA-4 cytoplasmic tail chimeric constructs did not associate with the p85 subunit of PI-3 kinase after stimulation with mAb. The analogous CD8-D28 cytoplasmic tail constructs did associate with PI-3 kinase after triggering. In contrast, Schneider et al. (21) showed that triggering with mAbs of native CTLA-4 in a T cell line led to PI-3 kinase association with CTLA-4 and activation of PI-3 kinase activity, similar to what has been reported for the CD28 receptor (22). Clearly more must be done to elucidate the signal transduction pathway(s) of CTLA-4, and to determine how this pathway(s) compares with that of CD28.

Over the last several years, the CD28/CTLA-4/CD80/CD86 receptor system has emerged as a key control point in the pathway(s) leading to T cell activation during immune responses. Blockade of this pathway leads to immunosuppression, prolongs organ graft acceptance, and ameliorates autoimmune disease in rodent models (23, 24); stimulation of this pathway can facilitate immune rejection of tumors (25). Now that the importance of this system has been established, we are beginning to unravel its molecular details. We have begun to appreciate that each of the players in this system has evolved unique characteristics that contribute to the exquisite regulation of this powerful system. The flurry of recent studies on the CTLA-4 receptor signifies the beginning of our understanding of the role of this fascinating molecule in T cell immune responses.

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