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Mechanisms of negative costimulation by CTLA-4 and its manipulation in tumor immunotherapy in mice and man

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Abstract

Activation of naive T cells is a complex and dynamic process involving not only recognition by the T-cell antigen receptor (TCR) but also additional costimulatory signals. Particularly crucial are the costimulatory signals mediated by the interaction of CD28 on the T cell with members of the B7 family of the antigen-presenting cell, and inhibitory signals mediated by interaction between CTLA-4 on the T cell with B7 on the APC. The dynamic integration of the TCR, CD28 and CTLA-4 signals determines the outcome of T-cell activation. There are key differences in CD28 and CTLA-4 that are relevant to their functional mechanisms: CTLA-4 has a much higher avidity for binding B7 family members than does CD28. CD28 is constitutively expressed on the plasma membrane while CTLA-4 is induced upon activation and is found largely in vesicles inside the cell. The latter properties suggest that its trafficking properties may serve to restrict its ability to inhibit T-cell responses. We have found that accumulation of CTLA-4 at the T cell-APC interface is proportional to the strength of the TCR signal, while recruitment of PKC and CD28 to the synapse is largely independent of agonist signal strength. This is consistent with functional data demonstrating that T cells receiving stronger stimuli are more susceptible to CTLA-4-mediated inhibition. This suggests a novel feedback control mechanism in which a stimulatory signal specifically regulates not only the transcription and expression of an inhibitory receptor, but also its recruitment to a functionally relevant site on the cell surface. This mechanism of attenuation suggests previously unrecognized roles for CTLA-4 in regulation of T-cell responses.

Over the past several years, we have demonstrated that blockade of the inhibitory CTLA-4 signals by administration of anti-CTLA-4 antibodies can greatly enhance T-cell responses *in vivo*. We have applied CTLA-4 blockade to a number of experimental tumor systems in mice. Administration of anti-CTLA-4 by itself is sufficient to induce rejection of many highly immunogenic tumors, including well-established tumors. Rejection is accompanied by long-lived immunity to tumor rechallenge. Tumors considered to be non-immunogenic, including the B16 melanoma and the SM1 mammary carcinoma, are resistant to treatment with anti-CTLA-4 alone. However, we have shown that administration of anti-CTLA-4 along with irradiated GM-CSF producing tumor cell vaccines results in prophylactic immunity and can lead to rejection of established tumors. In the case of B16 melanoma, rejection is accompanied by a progressive autoimmune depigmentation. The effect of anti-CTLA-4 in combination with the tumor cell vaccine in this setting is the induction of a potent CD8+ T-cell response directed against a melanocyte differentiation antigen TRP-2. Studies of the mechanism of the combination therapy have led to two very interesting observations. The first is that tumor rejection obtained by the combination therapy does not require the presence of CD4+ T cells, suggesting that blockade of CTLA-4 allows the protection of CD8+ T cells in a T helper cell independent manner. The second observation is that the anti-tumor response elicited by the combination CTLA-4 treatment is more potent in the absence of CD4+, and in particular

CD25+CD4+ Treg cells. This suggests that CTLA-4 and CD25+ Treg cells are independently regulating the anti-tumor response.

Antibodies to human CTLA-4 have been produced (Genpharm, Inc.) and Phase I clinical trials in both prostate cancer and melanoma are underway (Medarex). Results of these trials, which demonstrate the safety and anti-tumor activity of anti-CTLA-4, will be discussed.

References

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