

# Isolating tumor-reactive T cells and making them work in tumor therapy

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The development of molecular methods to identify candidate tumor antigens that might be targeted for attack by T cells, in concert with improved understanding of the requirements for T-cell activation and with advances in cell culture technology, has encouraged efforts to pursue adoptive T-cell therapy as a means to treat human malignancies. However, despite the characterization of many immunogenic tumor antigens, clinical trials with demonstrable therapeutic efficacy have been limited. Our lab has been addressing the obstacles to success with T-cell therapy, and has now resolved several major impediments to pursuing T-cell therapy as a modality to treating patients with established malignancies that should facilitate the broader application of this approach.

One major problem has been the difficulty generating and expanding tumor-reactive CD8+ T cells to sufficient numbers for tumor therapy. This has likely reflected the nature of the underlying host response to the targeted antigens - with the host either being naive to the tumor antigen or tolerant due to prior encounter with the antigen in the context of expression by normal tissues or by the progressing tumor. For generating primary *in vitro* responses, the use of dendritic cells as the antigen presenting cell coupled with sensitive techniques to enumerate responses has made it possible to detect responses to most candidate tumor antigens examined. Despite this encouraging result demonstrating *in vitro* priming, cloning and expanding the reactive cells, unlike the experience with *in vitro* expansion of effector memory cells previously primed *in vivo*, has proven surprisingly difficult, with the majority of cells undergoing apoptosis rather than proliferating with repeated stimulations. These events resemble the outcome of *in vivo* priming in murine models in the absence of CD4+ T cell help, in which short-lived effector cells are formed but memory cells are not developed and recall responses are not detected. Therefore, *in vitro* stimulation conditions have been evaluated to identify conditions that better mimic the events associated with effective *in vivo* priming of CD8+ cells with regard to the requisite cells and/or the supplements that should be present, and we have observed dramatic differences both in the numbers of reactive CD8+ cells induced following stimulation, as well as the ability to clone and expand such responses. Critical modifications have included the addition of exogenous IL-7 during the initial priming stimulation (the IL-7 receptor is expressed on memory precursors and not on effectors), and the addition of IL-15 during the expansion phase (memory T cells receive a pro-survival signal via the IL-15 receptor).

In the setting of tolerance to the candidate antigen, alternative obstacles are present - in particular tolerant cells have been rendered incapable of proliferating to antigen making *in vitro* sensitization and expansion not possible. To address this problem, we have created a transgenic mouse model in which the tumor antigen is also expressed in normal tissues, and a

second transgenic strain in which all CD8+ T cells express a TCR specific for the tumor antigen to provide an unlimited source of traceable high affinity T cells. Transgenic TCR cells that develop in the environment in which the tumor antigen is expressed in normal tissues can be isolated in large numbers - these cells have been tolerized, exhibiting characteristics affirming the previous antigen encounter but also lacking the ability to proliferate if stimulated *in vitro* with antigen. We have characterized these cells molecularly, using cDNA arrays to identify gene expression patterns that might contribute to maintaining the tolerant state, and phenotypically to directly assess expression of selected proteins. Based on this data set, we have identified a means to rescue and expand the tolerant cells, and demonstrated that the rescued cells can be used in therapy of an established tumor.

In clinical settings in which tumor-reactive T cells have been successfully isolated, expanded, and infused into patients, the therapeutic results have often been somewhat disappointing, with only a subset of patients responding and a smaller fraction achieving durable remissions. Although this has occasionally resulted from antigen escape, with outgrowth of tumor variants that no longer express the targeted antigen, the basis for failure in the majority of cases has been more elusive. However, studies in murine models as well as analyses of cancer patients have identified many potentially important contributing factors, including induction of anergy by chronic antigen stimulation *in vivo*, poor immunogenicity of tumor cells due to absence of costimulatory molecules and/or down-regulation of the antigen processing machinery, and inhibition of the activity of tumor-reactive T cells by factors secreted by the tumor such as TGF-beta or by the increased numbers of Treg cells found in cancer patients. Therefore, achieving reproducibly effective therapy will likely require providing cells that can overcome such hurdles, and we have examined genetic modification of T cells as a means to create CD8+ T cells that can retain activity in this "hostile environment". As an alternative to introducing genes that impart individual effector functions, strategies are being explored to disrupt the activity of negative regulatory pathways that function intracellularly to attenuate T-cell activation and responsiveness, as these pathways globally impact T-cell functions. Studies abating via introduction of dominant negative proteins or targeted siRNA molecules the activity of Cbl-b, an adapter protein in the E3 Ubiquitin ligase family that functions to modulate levels/activity of the TCR complex and critical proximal signaling molecules responsible for Ca<sup>++</sup> flux, such as PLC-gamma and costimulation such as PI3-kinase, will be discussed.

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