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Commentary

Cancer Immunology Research

# Cytotoxic T-cell Cytokines Put Cancer Under Arrest

Stanley R. Riddell

*See related articles by Matsushita et al., p. 26, and Acquavella et al., p. 37*

Cancer immunotherapy using monoclonal antibodies that block inhibitory signaling molecules expressed on T cells and the adoptive transfer of T cells isolated from tumor infiltrates or engineered with T-cell receptors (TCR) or synthetic chimeric antigen receptors that target tumor-associated antigens has emerged as an effective modality for a variety of human malignancies (1–9). $CD8^+$ and $CD4^+$ T cells are ideally suited to attack tumors because they can specifically recognize mutant and overexpressed antigens presented by tumor cells, proliferate *in vivo*, and employ an impressive armamentarium of effector mechanisms to promote tumor-cell destruction. $CD8^+$ cytotoxic T lymphocytes (CTL) release cytolytic granules containing perforin and granzymes, engage death receptors that induce tumor-cell apoptosis, and produce cytokines that can be directly cytotoxic and recruit other effector cells to the tumor site (10, 11). In some settings, the adoptive transfer of comparatively small numbers of tumor-specific T cells is accompanied by their marked proliferation in response to tumor-cell recognition and the complete regression of large and widely disseminated tumor-cell masses, leading to the conclusion that T cells are highly efficient serial killers (11, 12). However, it is not uncommon that tumor regression after immunotherapy is temporally delayed and not associated with easily detectable tumor-specific T cells in the blood or evidence of their marked proliferation at the tumor site. Indeed, a significant subset of patients exhibit prolonged periods of "stable disease" after immunotherapy during which tumors persist but growth is either noticeably slower or halted, which has required revision of response criteria for this therapeutic modality (13, 14). The mechanisms for delayed responses and stable disease are not well defined and run counter to the perception that tumor-cell destruction results primarily from a "kiss of death" by $CD8^+$ CTLs engaging tumor cells and releasing cytotoxic granules or engaging death receptors (15, 16).

Prior studies in murine models have demonstrated that effector mechanisms distinct from direct cytolysis of tumor cells contribute to immune-mediated control of cancer, potentially explaining how in some settings relatively few tumor-specific T cells can successfully destroy or delay progression of large tumors. For example, $CD8^+$ T cells have effects on tumor-supporting stromal cells by recognizing cross-presented tumor antigens and damage the vasculature essential for providing the nutrients that sustain tumor-cell survival and proliferation (17–20). These indirect mechanisms are operative in promoting regression of very large established tumors and may be critical for preventing the outgrowth of tumor-cell variants that might escape T-cell recognition due to lack of expression of the MHC-restricting allele, defects in antigen processing, or loss of antigen expression. The susceptibility of tumor stromal cells and vasculature to $CD8^+$ T cells has been shown to require their expression of IFNγ and TNF receptors, and local production of IFNγ and TNFα by T cells, but is not dependent on expression of perforin (17, 19). The induction of tumor-cell senescence by IFNγ and TNFα produced by tumor-specific $CD4^+$ helper T cells has recently been shown in a model of pancreatic beta cell tumors induced by transgenic expression of the SV40 T antigen under the insulin promoter (21). In this model, IFNγ and TNFα produced locally by $CD4^+$ T cells activated STAT 1 in tumor cells that mediated a permanent growth arrest through JUNB and p16Ink4a (21). Human tumor cell lines and primary melanoma cells were shown similarly to undergo growth arrest when exposed to IFNγ and TNF *in vitro*, suggesting that a cytokine-induced growth arrest may be a general mechanism by which tumor-specific $CD4^+$ T cells inhibit tumor progression.

Two articles in this issue of *Cancer Immunology Research* examining mechanisms of $CD8^+$ T-cell antitumor activity provide further support for direct effects of IFNγ and TNFα in arresting tumor-cell proliferation and identify pathways in addition to p16Ink4a that mediate this effect. Matsushita and colleagues used gene expression profiling of implanted B16 melanoma tumors after the adoptive transfer of $CD8^+$ T cells and found that cell-cycle–promoting genes, including *Skp2*, *E2f2*, *Ccnf7*, *Mki67*, and *Wee1*, were rapidly downregulated in tumors treated with T cells compared with those of controls (22). Using an elegant model in which a fluorescent ubiquitination-based cell-cycle reporter was introduced into implanted B16 cells to enable imaging of cells in distinct stages of the cell cycle (21), the authors demonstrated that adoptive transfer of tumor-specific $CD8^+$ T cells resulted in a rapid shift from proliferating tumor cells to those arrested in $G_1$. Infiltration of T cells into the tumor was modest, and evidence of direct tumor lysis, although present, was restricted to very localized areas. Administering an anti-IFNγ antibody under conditions that did not affect the numbers of T cells infiltrating the tumor prevented the cell-cycle arrest in tumor cells. *In vitro* studies confirmed that culturing B16 tumor cells in IFNγ induced a $G_1$ arrest and revealed a synergistic effect of adding TNFα. Mechanistic studies revealed the expected activation of STAT1 in tumor cells and the downregulation of Skp2 and upregulation of the CDK inhibitor p27Kip*1*, both of which have been shown previously to correlate with cell-cycle arrest in tumor cells (23, 24). Thus, B16 melanoma, which has disabling mutations in the *p16Ink4* gene that were linked to tumor cell-cycle arrest mediated by $CD4^+$ T cells (21), remains susceptible to the growth arrest

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effects of IFNγ, demonstrating that more than one pathway downstream of STAT1 can be activated to promote tumor cell-cycle arrest.

In a separate study, Acquavella and colleagues examined potential mechanisms for synergy of adoptively transferring $CD8^+$ T cells together with drug therapy that targets the $BRAF^{V600E}$ oncogene in a murine model (25). $BRAF^{V600E}$ is expressed in over 50% of human melanoma and promotes tumor growth through constitutive activation of the MAPK pathway (26). Inhibition of $BRAF^{V600E}$ with vemurafinib induces dramatic tumor regression in patients with advanced melanoma, but drug resistance develops and unfortunately the antitumor responses are transient (26–29). Adoptive transfer of tumor-infiltrating T cells also has therapeutic effects in a subset of patients with melanoma, and prior studies have suggested that adoptive T-cell therapy or checkpoint blockade might be effectively combined with $BRAF^{V600E}$ inhibition (30–33). To explore this possibility and examine potential mechanisms of synergy, Acquavella and colleagues developed a murine $BRAF^{V600E}$-mutant melanoma cell line that, when transplanted into wild-type mice, recapitulates the responsiveness and development of vemurafinib resistance that are observed in human melanoma. The tumor cells were transfected to express murine gp100 recognized by pmel-TCR transgenic $CD8^+$ T cells to enable evaluation of the combination of vemurafinib with adoptive T-cell therapy. The results demonstrated synergistic *in vivo* antitumor activity of combined therapy with vemurafinib and T cells, which unexpectedly was not due to improved recruitment or effector function of transferred T cells but rather to enhanced arrest of tumor-cell proliferation. This growth arrest could be recapitulated *in vitro* by combining vemurafinib with IFNγ and TNFα. Transcriptional profiling of tumor cells exposed to vemurafinib, IFNγ and TNFα, or both indicated that vemurafinib increased the IFNγ/TNFα gene signature, suggesting that tumor cells would be more sensitive to the antiproliferative effects of these cytokines.

The interactions between immune cells, tumor cells, and cytokines in the tumor microenvironment are increasingly being scrutinized in an effort to identify subsets of patients who will be responsive to immunotherapy, and to identify obstacles in patients who do not respond (34–36). The studies of Matsushita and colleagues (22) and Acquavella and colleagues (25) show that it may not be necessary for large numbers of T cells to infiltrate and directly lyse tumors to have profound therapeutic effects because of the effects of secreted molecules that diffuse in this complex environment. The ease with which T cells can be genetically modified suggests that expressing genes that encode secreted molecules that are capable of inhibiting tumor cells directly, or targeting key functions of stromal, vascular, or other immune cells that promote tumor progression, is a viable approach to enhance the therapeutic activity of adoptive therapy (37).

IFNγ has multiple activities that favor tumor elimination by the immune system, including upregulating expression of MHC and antigen-presentation molecules, induction of chemokines that recruit effector cells and hinder neovascularization, and antiproliferative effects on tumor cells. A key question raised by the studies reported here is whether the antiproliferative effects of IFNγ are sufficiently specific, potent, and durable to improve the success of immunotherapy in the clinic. Disappointingly, the observed cell-cycle arrest induced in B16 melanoma by T cells in the animal model appears to be relatively short lived, and the mechanisms of tumor escape are not informed by the current studies. Possibilities include cessation of IFNγ production by T cells rendered dysfunctional in the tumor microenvironment, or selection of tumor cells that are resistant to IFNγ and escape the induced cell-cycle arrest. Although a role for IFNγ in these models is clear, it is also true that this cytokine has many effects that promote tumor progression, including upregulation of PD-L1, increasing myeloid-derived suppressor cells and regulatory T cells, and inducing idolamine 2,3 dioxygenase (38). Understanding the context in which adoptive T-cell therapy and/or checkpoint blockade increases the local levels of cytokines that arrest tumor growth, the nature of the microenvironment and the adaptive mechanisms that are induced will be critical to fully exploit potential therapeutic effects. Combining pharmacologic suppression of growth-promoting oncogenes with adoptive T-cell transfer is an initial step in this direction that can be evaluated in the clinic, and other combinations that take advantage of cytokine-induced tumor-cell growth arrest will likely emerge. However, as in law enforcement, it is not sufficient to simply arrest the perpetrator, but to collect enough evidence to get a conviction.

## Disclosure of Potential Conflicts of Interest

S.R. Riddell reports receiving commercial research support and has ownership interest (including patents) in Juno Therapeutics. He is also a consultant/advisory board member for Juno Therapeutics and Cell Medica.

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