Non-replicating Toxoplasma gondii reverses tumor-associated immunosuppression

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We examined the efficacy of using attenuated non-replicating Toxoplasma gondii uracil auxotrophs that can be safely delivered as anticancer immunotherapeutics. This strategy exerted remarkable therapeutic activity in murine models of melanoma and ovarian carcinoma, and holds broad potential for the development of novel, highly effective anticancer vaccines.

Tumors promote their own growth and evade immunosurveillance. The ability of malignant cells to establish immunosuppressive conditions plays a major role in tumor progression by interfering with immunological control mechanisms. The precise nature of tumor-associated immunosuppression varies with cancer type, is biologically complex, and often involves perturbation of both innate and adaptive mechanisms that would otherwise eradicate malignant lesions.¹

While the use of pathogenic microorganisms in cancer immunotherapy is not novel, protozoan parasites are relatively unexplored in this context. Toxoplasma gondii is an obligate intracellular protozoan parasite that actively invades host cells. Toxoplasma cells preferentially contact and invade myeloid cells including dendritic cells and monocytes/macrophages,² which are frequently involved in tumor-elicted immunosuppression. By invading myeloid cells, T. gondii gains direct access to the control of innate immune cells, generally resulting in the elicitation of potent Th1 immune responses.³ During invasion, Toxoplasma cells secrete a repertoire of specialized molecules that function to seize control of the host cell from within.³ The parasite also hijacks bystander cells as the molecules that it produces are injected into cells that are contacted but are not invaded.⁴ For example, T. gondii injects the rhoptry (ROP)16 kinase into macrophages, suppressing the signal transducer and activator of transcription 3 (STAT3)-dependent production of interleukin-12 (IL-12), and upregulating arginase 1 upon the activation of STAT6.⁵ Toxoplasma cells also secrete ROP18, a kinase that protects the vacuole housing intracellular parasites from innate immune attack mechanisms mediated by a family of interferon γ (IFNγ)-activated GTPases.⁶ Additional molecules secreted by T. gondii play significant roles in manipulating host cells and immune responses.³

A safe, live-attenuated, non-replicating variant of T. gondii was created as an uracil auxotroph (cps) strain.⁷ While uracil auxotrophs normally invade mammalian cells, they do not replicate in the absence of uracil, thus exhibiting an exquisite degree of attenuation of virulence in both normal and severely immunodeficient mice.⁷ In contrast to many prokaryotic microbes, the eukaryotic T. gondii does not harbor any significant toxin or otherwise toxic molecule. We therefore reasoned that strongly polarized Th1 host responses driven T. gondii-secreted factors and the manipulation of innate immune cells by means of the cps strain would stimulate responses in the tumor microenvironment that could break tumor-associated immunosuppression.

Immature CD11c⁺ dendritic cells accumulate in high amounts within solid epithelial tumors including ovarian carcinomas, and deliver signals that create a highly immunosuppressive microenvironment.⁸ Treatment of established aggressive vascular endothelial growth factor (VEGF)-expressing ID8 ovarian tumors with the cps strain resulted in tumor regression and improved the survival of tumor-bearing mice.⁹ Of note, the administration of the cps strain was equally effective in naïve mice as well as in mice that were immune to Toxoplasma. The immunotherapeutic effects of cps cells was completely dependent on IL-12, but not on Toll-like receptor (TLR) adaptor myeloid differentiation 88 (MYD88).⁹ In the tumor microenvironment as well as ex vivo, the cps strain preferentially invaded CD4⁺CD11c⁻ cells and both cps-infected and cps-contacted cells exhibited increased expression levels of the co-stimulatory molecules CD80 and CD86. The treatment of ovarian carcinomas with the cps strain rapidly reversed tumor-associated immunosuppression and stimulated the priming of CD8⁺ T-cell responses by antigen-presenting cells.⁹
Tumor antigen-specific CD8+ (and granzyme B+) T cells were increased both in the spleen and in the tumor microenvironment upon the administration of the cps strain,9 and adoptive transfer experiments demonstrated that T cells from treated mice potently suppressed the development of ovarian carcinomas.9 The cps strain also stimulated the recruitment of numerous cell types to neoplastic lesions and to the spleen. Of note, while the TH17+ cells were not increased by our immunotherapeutic approach, the percentage of intratumoral regulatory T cells (CD4+FOXP3+ T cells) was significantly decreased.

Along similar lines, the administration of the cps strain elicited the immune system-mediated regression of established B16F10 melanomas.10 More than 90% of cps-treated mice survived B16F10 melanoma and most of these animals developed localized and/or systemic vitiligo, as indication of the recognition of melanocytes by the immune system. Of note, while the T17+ cells were not increased by our immunotherapeutic approach, the percentage of intratumoral regulatory T cells (CD4+FOXP3+ T cells) was significantly decreased.

Figure 1. Active invasion by Toxoplasma gondii reverses tumor-elicited immunosuppression and activates tumor-targeting immune responses. Immunosuppressive myeloid CD11c+ cells in the tumor microenvironment are preferentially invaded by the non-replicating cps strain of T. gondii. Invaded or contacted myeloid cells are activated to produce interleukin-12 (IL-12) and to express increased levels of the co-stimulatory molecules CD80 and CD86 on their surface. The cps strain induces the maturation of myeloid CD11c+ cells, leading to increased antigen-presentation and T-cell priming. Eventually this results in the expansion of activated natural killer (NK) cells and CD8+ T lymphocytes, which release interferon γ (IFNγ) into the tumor microenvironment. Cellular responses such as those mediated by NK cells and tumor-specific CD8+ T lymphocytes mediate the killing of cancer, hence causing tumor regression.

A major advantage of cps-based immunotherapy is its versatility. The cps strain was originally developed as a self-adjuvant platform for stimulating potent T1 immune responses to engineered CD8+ T-cell vaccines.7 T. gondii uracil auxotrophs can be easily engineered with conventional genetic techniques to exacerbate vaccine-elicited immune responses, to express specific molecules (or exert selected functions) in the tumor microenvironment, or to selectively target particular cell types. These versatile biological features along with the inherent and potent immunotherapeutic potential of the cps platform itself open multiple avenues and a wide-range of potential applications. Exploiting the unique biological features of the safe Toxoplasma uracil auxotroph vaccine platform is expected to drive the development of innovative cancer vaccines that are able to eradicate established lesions as well as prevent disease recurrence.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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