Published in final edited form as:

Trends Parasitol. 2013 September ; 29(9): 431–437. doi:10.1016/j.pt.2013.07.001.

Targeting tumors with nonreplicating *Toxoplasma gondii* uracil auxotroph vaccines

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Abstract

Toxoplasma gondii is an intracellular parasite that has evolved to actively control its invaded host cells. *Toxoplasma* triggers then actively regulates host innate IL-12 and interferon- responses that elicit T cell control of infection. A live, nonreplicating avirulent uracil auxotroph vaccine strain (*cps*) of *Toxoplasma* triggers novel innate immune responses that stimulate amplified CD8⁺ T cell responses and life-long immunity in vaccinated mice. Here, we review recent reports showing that intratumoral treatment with *cps* activated immune-mediated regression of established solid tumors in mice. We speculate that a better understanding of host-parasite interaction at the molecular level and applying improved genetic models based on *ku80* *Toxoplasma* strains will stimulate development of highly effective immunotherapeutic cancer vaccine strategies using engineered uracil auxotrophs.

Keywords

Toxoplasma gondii; avirulent uracil auxotrophs; IL-12p70; CD8⁺ T cells; immunotherapy; tumor regression

Toxoplasma as a cancer immunotherapeutic agent

Toxoplasma gondii is an obligate intracellular protozoan parasite with an extremely broad host range inclusive of most birds and virtually all mammals [1], including approximately one-third of the human population [2]. Three main strain types (I, II, and III) are present in North America and Europe, and these strain types differ with respect to their virulence traits in mice [3]. Type I strains are highly virulent, type II have low virulence, and type III strains are avirulent. While *Toxoplasma* causes particularly severe infections in the context of immune deficiency or during congenital infection [1], the majority of *Toxoplasma* infections in humans are asymptomatic. *Toxoplasma* infection is initiated by oral ingestion of infectious parasite stages (cysts or sporozoites) present in contaminated food or water [4]. Following transmission to a new host, the parasite differentiates into the acute stage form (tachyzoite) and then rapidly disseminates [5]. The host immune system responds vigorously to *Toxoplasma* infection and gains control of the acute infection (reviewed in [6–8]). The biological aim of *Toxoplasma* is to establish a chronic infection that increases the chance for subsequent transmission of the parasite to a new host [5, 6].

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Cancer immunotherapy involves the use of methods or agents to stimulate immune responses that can regress tumors (reviewed in [9–13]). Many of the cytokine, chemokine, and cellular immune responses elicited by *Toxoplasma* [6–8] parallel immune responses associated with significant antitumor immune responses. For example, *Toxoplasma* infection elicits significant IL-12 production [6, 8]. IL-12 used in tumor immunotherapy induces Th1 differentiation and reprograms dysfunctional tumor-associated T cells [14]. IL-12 also amplifies T cell and NK cell responses [15, 16], inhibits angiogenesis [17], and manipulates Th1 cells to promote the differentiation of macrophages to the M1 phenotype, found to exhibit superior antitumor responses [18, 19].

Clinical trials have shown that direct IL-12 therapy increases infiltration of macrophages and NK cells [20, 21] and, more importantly, CD8⁺ T cells to tumor sites [22]. Tumor-specific CD8⁺ T cells are potent effectors that directly target tumor cells for killing [23]. While tumors create immune suppressed environments that reduce T cell functions [9, 13], IL-12 restores cytolytic function to tumor-resident CD8⁺ T cells resulting in effective tumor killing [9]. Unfortunately, direct delivery of IL-12 in tumor immunotherapy has been associated with significant systemic toxicity [24, 25].

Microbes have long been explored as potential cancer immunotherapeutic agents. For example, *Toxoplasma* was used more than 5 decades ago to demonstrate anti-tumor effects [26]. Protein extracts from killed *Toxoplasma* demonstrated measurable effects in slowing the development of various tumors such as fibrosarcoma [27], lung [28], melanoma [29], sarcoma [30, 31], and chemically-induced tumors [32]. Other studies have used live *Toxoplasma* to demonstrate measurable effects in slowing the development of bladder [33], brain [34], leukemia [35], Lewis lung [36], melanoma [37, 38], and sarcoma [35] tumors in mice. However, these studies were limited by the virulence of natural *Toxoplasma* infection in mice. Nonetheless, macrophages were implicated to play an antitumor role in several of these studies [33–35]. Recent studies attributed the antitumor effect to Th1 responses, IFN- γ , or inhibition of angiogenesis [36–38]. Unfortunately, these studies did not demonstrate therapeutic effects against established tumors. In addition, live replicating strains of *Toxoplasma* are unlikely to be suitable for use in tumor therapy due to the fact that cancer patients are immunocompromised and are thus more vulnerable to infection. Therefore, attenuated *Toxoplasma* strains such as the nonreplicating uracil auxotroph vaccine strain (*cps*) may be more useful for antitumor therapy [39].

The *Toxoplasma cps* vaccine strain

Disruption of the first step in the *de novo* pyrimidine synthesis pathway by targeted insertional mutagenesis of the carbamoyl phosphate synthetase II gene created a mutant strain (*cps*) that exhibited uracil auxotrophy and extreme attenuation of virulence [39]. The *cps* strain invades and replicates normally *in vitro* if uracil is supplied in the culture medium. However, uracil is not present at any significant concentration in mammals or their cells, and correspondingly the *cps* strain invades host cells normally but does not replicate in the host cell or in mice and thus exhibits an extraordinary decrease of greater than 7 logs of virulence in mice [39, 40]. The *cps* vaccine strain is also avirulent in immune deficient mice that do not express IFN- γ [39]. Immune competent mice vaccinated with *cps* produce a potent protective immunity against rechallenge with virulent type I strains or chronic type II strains [39–41]. Vaccination of mice with *cps* is successful by several routes [40], and this vaccine elicits life-long immunity, which is based on the generation of CD8⁺ T cell memory [40]. Protective CD8⁺ T cell immunity is elicited by *cps* even in MyD88 deficient mice [42]. Still, the immunity elicited by *cps* vaccination relies on the ability of mice to express IL-12 [42] and IFN- γ [39].

The innate response to *cps* vaccination with respect to IL-12 biology is unusual. While the virulent type I RH parental strain of *cps* produces delayed and low-level production of IL-12 at the site of infection, and systemically, the avirulent *cps* strain elicits a rapid and sustained high-level production of IL-12 [40]. This result is surprising because the IL-12 response to the type I *cps* vaccine reflects the pattern observed in less virulent, naturally occurring type II strains [40, 43]. Remarkably, IL-12 produced by *cps* vaccination was found in the form of mature IL-12p70 [40], a key molecule in effectively bridging innate and adaptive immunity. The induction of IL-12p70 by *cps* is rapid, and this high-level production of IL-12p70 persists for at least one week both at the local *cps* vaccination site and systemically [40].

The pattern of IFN- γ production by *cps* also differs from its virulent RH parent. While production of IFN- γ in response to *cps* was significant at the local vaccination site, IFN- γ levels were observed to be transient or absent systemically [40]. By contrast, the RH strain produces extremely high systemic levels of IFN- γ , reflecting significant systemic inflammation. Surprisingly, vaccination with *cps* stimulates more effective activation of IFN- γ -mediated macrophage killing mechanisms that effectively suppresses the replication of virulent type I strains [44].

The *cps* vaccine model has been useful in identifying the role of IL-12 signaling in shaping the differentiation and development of the protective CD8⁺ T cell responses [45–47]. The *cps* vaccine model is amenable for engineering useful vaccine strategies to generate CD8⁺ T cell immunity not only against *Toxoplasma*, but also perhaps against other pathogens or tumors [39, 40, 48]. For example, heterologous expression of the model antigen ovalbumin (OVA) by *cps* engineered to secrete OVA into the parasitophorous vacuole space resulted in high-level OVA antigen-specific CD8⁺ T cell responses [49]. Approximately 30% of the peritoneal CD8⁺ T cell population specifically recognized the SIINFELK OVA OT-I peptide following intraperitoneal vaccination with *cps*-OVA [49]. These observations indicate that the *cps* vaccine provides both a vehicle for expression of foreign T cell vaccine components, as well as self-adjuvant properties that stimulate CD8⁺ T cell vaccine responses. Furthermore, OVA-specific CD8⁺ T cells elicited by *cps*-OVA vaccination were fully functional. These OVA antigen-specific CD8⁺ T cells expressed cytolytic granzyme B and perforin, and specifically targeted the killing of OVA-expressing target cells *in vitro* and *in vivo* [49]. While CD4⁺ T cells contribute to the priming of CD8⁺ T cells [49], a significant antigen-specific CD8⁺ T cell responses was still generated by *cps* without CD4⁺ T cell help [49], suggesting that vaccination with *cps* may be at least partly successful even in immune deficient populations. High-level CD8⁺ T cell responses elicited by *cps* may arise from optimal antigen presentation and the rapid bridging of innate and adaptive responses by early IL-12p70 production [40].

Since William Coley's original studies in 1891 that used *Serratia* and *Streptococcus* to attempt remission of sarcoma [50], active stimulation and durable immunity to tumors remains elusive. The *cps* vaccine strain elicits strong Th1-biased immune responses that may be useful in cancer immunotherapy [9, 13]. Vaccination with *cps* promotes cellular recruitment [40], local and systemic immune activation [40, 49], rapid and sustained high-level IL12p70 production [40], IFN- γ and innate cytokine and chemokine production [40], high-level IFN- γ -dependent activation of macrophages [51], and exceptional CD8⁺ T cell responses [39, 40, 49]. The development of a Th1-biased immune response with expression of IL-12, IFN- γ and CD8⁺ T cell responses is associated with more effective control and eradication of various tumors [9, 13, 52]. Thus from these earlier studies there was a strong indication that *cps* might have the ability to reduce tumor-associated immune suppression to promote the development of antitumor CD8⁺ T cell responses.

Treatment of melanoma with *cps*

Melanoma is a malignant tumor of melanocytes. Despite recent progress in treatment strategies, melanoma is still the most frequently lethal skin cancer. Consequently, cancer immunotherapies for metastatic melanoma have been explored as a potential treatment. By example, CD8⁺ T cells have been shown to provide therapeutic benefits in the treatment of melanoma [53, 54].

B16F10 melanoma provides a commonly used, highly aggressive and poorly immunogenic model for murine tumor immunotherapy studies [55]. While combinations of immune therapies have achieved regression of B16F10 tumors [11, 56], immune-based monotherapy of aggressive B16F10 melanoma has not been successful in mediating tumor regression. Recently, the *Toxoplasma cps* strain was examined as an immunotherapeutic treatment for established B16F10 melanoma. Intratumoral treatment of B16F10 melanoma with *cps* elicited immune-mediated regression of established primary tumors [57]. Remarkably, *cps* treatment is the first monotherapy that demonstrated effective regression of primary B16F10 tumors in mice [57]. While a single administration of *cps* was not sufficient to promote tumor regression, high-dose intratumoral *cps* treatment on days 10, 11, 14, and 15 after B16F10 cells were transplanted intradermally elicited highly effective regression of primary melanoma. In 100% of *cps*-treated mice, the tumor initially halted its growth after *cps* treatment, the melanoma then rapidly regressed, and tumors were no longer detectable within 12 days after treatment [57]. More than 90% of *cps*-treated melanoma-bearing mice survived long-term without tumor recurrence. A significant fraction of surviving mice (76%) developed localized and/or systemic vitiligo, a predictor of positive outcomes in melanoma patients, that indicates the likelihood that CD8⁺ T cell responses specific to melanocytes was generated by *cps* treatment [57].

Therapy by *cps* depended on an intact immune system since treatment failed in NOD/SCID/IL-2R⁻ chain knockout mice that lack B, T, and NK cells [57]. The *cps* immunotherapy depended on CD8⁺ T cells and on NK cells, but success of the treatment did not require the presence of CD4⁺ T cells. The *cps*-mediated melanoma immunotherapy was dependent on IL-12 and IFN- γ expression as the lack of either cytokine ablated the therapy. The *cps*-mediated melanoma immunotherapy was also partially dependent on expression of the CXCR3 chemokine receptor that is expressed on T cells. Correspondingly, *cps* treatment elicited rapid and sustained intratumoral production of CXCL9 and CXCL10, chemokines that attract CXCR3⁺ T cells [58]. Interestingly, CXCL10 was also recently reported to promote more effective motile targeting of CD8⁺ T cells to their *Toxoplasma*-infected targets [59].

Extended heat-inactivation of *cps* (65°C) eliminated the therapeutic effect of *cps* treatment, suggesting that live, invasive *cps* may be necessary for treatment efficacy. During parasite invasion, *Toxoplasma* injects a number of secreted effector molecules (such as ROP5, ROP16, and ROP18) into its host cells to manipulate host cell behavior [6, 60–63].

Toxoplasma also triggers toll-like receptors (TLR) [8]. It cannot be yet ruled out that this extreme heat treatment may have inactivated molecules that trigger TLR [8], or inactivated secreted parasite molecules that manipulate host cells from within [6]. Thus additional studies are still needed to clearly define the role for secreted parasite molecules or active invasion in this *cps*-mediated immunotherapy [57].

Examination of tumor-associated cells showed that *cps* recruited and invaded many cell types in the tumor environment [57]. Between 60% and 80% of the tumor-associated CD45⁺, CD11b⁺CD11c⁻, CD11b⁺CD11c⁺, and NK1.1⁺ cells were actively invaded by *cps*. In addition, between 25% to 50% of CD45⁻, CD3⁺, and GR1⁺CD11c⁻ cell types were also

actively invaded by *cps*. These observations suggested that *cps* primarily invaded macrophages, dendritic cells, and NK cells, but also gained significant access to T cells, neutrophils, and CD45⁻ (nonhematopoietic) cell types, which were primarily tumor cells. Within 18 hours after the second *cps* treatment on day 11, the percentage of CD45⁺, CD11b⁺CD11c⁻, and CD3⁺ cells significantly increased in the tumor, although CD11b⁺CD11c⁺, NK1.1⁺, and surprisingly GR1⁺CD11b⁻ cells (neutrophils) did not increase in their percentage in the tumor. Within 3–4 days following *cps* treatment, the percentage of CD45⁺ cells, CD8⁺ T cells, IFN- γ -expressing CD8⁺ T cells, and IFN- γ -expressing NK1.1⁺ cells significantly increased in the tumor. By eight days post-treatment, antigen experienced CD44⁺CD8⁺ T cells and CD44⁺CD4⁺ T cells were increased in their percentage in the spleen and at the draining lymph node. Furthermore, the percentage of IFN- γ -expressing CD8⁺ T cells specific to the melanoma TRP-2 antigen was significantly increased by *cps* treatment [57]. These results indicated that *cps* elicited a potent IL-12 and IFN- γ -innate response that led to the activation of melanoma specific CD8⁺ T cells. In addition, rechallenge experiments on surviving mice demonstrated that *cps* treatment generated detectable but not completely durable protective memory responses to melanoma [57].

Treatment of ovarian cancer with *cps*

Early-stage detection of ovarian cancer is difficult. Consequently, later-stage tumors are often present at the time any treatment begins. Ovarian cancer patients typically respond well to the initial cancer treatments, but the incidence of cancer recurrence remains high, and successful treatment becomes more difficult. Survival rates for patients with ovarian cancer have changed little in the last 30 years [64] showing the dire need for both improved diagnosis (and awareness), as well as improved treatment modalities.

Ovarian cancer creates a highly immunosuppressive tumor environment at tumor locations [65–67]. Immature CD11c⁺ dendritic cells accumulate to high numbers in solid epithelial ovarian tumors and deliver proangiogenic and immunosuppressive mediators [65, 68, 69]. Ovarian cancer actively suppresses professional antigen presenting cell priming of CD8⁺ T cells by cross-presentation [65], and also actively suppresses T cell functions in the tumor environment [70]. While antitumor responses are naturally elicited early during tumor development [71, 72], these antitumor responses decrease as the tumor environment becomes increasingly immunosuppressive [73]. Consequently, any effective ovarian cancer immunotherapy must overcome the highly immunosuppressive environment of the ovarian tumor [13]. By example, stimulation of ovarian tumor-associated CD11c⁺ dendritic cells elicited antitumor responses that delayed tumor progression in mice [65, 70, 74, 75].

There are several murine models of ovarian cancer based on the ID8 ovarian tumor cell line [76] that differ with respect to how aggressively the tumor develops. ID8 cells engineered to express vascular endothelial factor-f (ID8- *Vegf*) accelerate vascularization (angiogenesis) of the tumor, and mice succumb to ovarian cancer in 50 to 70 days [77], while mice succumb to the parental ID8 tumor in approximately 100 days [78]. Engineering of the ID8- *Vegf* model to also express α -defensin (a recruitment factor for dendritic cells) created the hyper-aggressive ID8- *Vegf/Defb29* model [65] that promotes rapid ovarian tumor development (mice succumb in ~ 40 days).

We recently examined *cps* immunotherapy in the aggressive ID8- *Vegf* and the hyper-aggressive ID8- *Vegf/Defb29* tumor models in mice. We discovered that intraperitoneal treatment of established aggressive ID8- *Vegf* ovarian tumors with *cps* regressed tumors, and treated mice survived long-term (> 1 year) [79]. The protocol for this *cps* immunotherapy involved two low-dose intratumoral *cps* treatments that were delivered 12 days apart. No

previous immunotherapy has demonstrated complete tumor regression in the ID8- *Vegf* ovarian tumor model. Intratumoral *cps* treatment of established hyper-aggressive ID8- *Vegf/Defb29* ovarian tumors provided a substantial therapeutic benefit, although these ovarian tumors were not cleared by a two-dose *cps* treatment. Remarkably, the *cps* immunotherapy was equally effective in previously vaccinated mice that were already immune to reinfection with *Toxoplasma* [57, 79]. Thus previous exposure to *Toxoplasma*, as is common in humans, did not reduce the efficacy of the *cps* treatment. Table 1 summarizes tumor treatment studies that have been reported using the nonreplicating *cps* strain of *T. gondii*.

The therapeutic benefit of *cps* treatment in ovarian cancer was dependent on the expression of IL-12 [79]. Correspondingly, intratumoral IL12p40 and IL-12p70 were rapidly elicited following *cps* treatment. IL-12 has the potential to inhibit angiogenesis [17]. Remarkably, the therapeutic benefit of *cps* treatment in ovarian cancer was not dependent on expression of MyD88, suggesting that MyD88-linked TLR is dispensable for the success of *cps* immunotherapy of ovarian carcinoma.

Cell types invaded by *cps* in the ovarian tumor environment were tracked by phenotype and function [79]. The antigen-presenting CD11c⁺ dendritic cell is the predominant CD45⁺ cell type in the ovarian tumor environment [65, 75]. We found the CD45⁺ cells accounted for the vast majority (86%) of *cps*-invaded cell types in the tumor environment, and 79% of the CD45⁺CD11c⁺ antigen-presenting cell types were actively invaded by *cps* within 18 hours after treatment. CD45⁺CD11c⁺ cells were preferentially invaded 14-fold more frequently than CD45⁺CD11c⁻ cells. CD45⁺CD11c⁺ cells in the untreated tumor environment expressed only modest levels of CD86 and low levels of CD80, costimulatory molecules essential for the activation of T cell immunity. Within 18 hours after *cps* treatment the CD45⁺CD11c⁺ cells present in the tumor environment expressed high-level activation of both CD86 and CD80 molecules on their surface. While there was increased recruitment of new CD45⁺CD11c⁻ cells to the ovarian tumor environment, *ex vivo* studies demonstrated that *cps* treatment directly activated maturation of immune suppressed CD45⁺CD11c⁻ cells present in the tumor environment [79]. Additionally, both *cps*-invaded as well as *cps*-exposed (noninvaded bystander CD45⁺CD11c⁻ cells) CD45⁺CD11c⁻ cells increased expression of CD80 and CD86, although *cps*-invaded cells expressed higher levels of both maturation markers, and high-level expression of CD86 was only observed in invaded cells [79]. This observation is interesting in view of recent reports that have shown that *Toxoplasma* has the ability to also inject secreted effector molecules into contacted but not invaded cells [80, 81]. High-level expression of CD86 was previously reported in *Toxoplasma*-invaded macrophages [82], and *Toxoplasma*-invaded dendritic cells previously have been reported to significantly upregulate markers of maturation [83]. Collectively, these observations suggested that *cps* treatment triggered immune activation of the ovarian tumor environment. To functionally examine this possibility, antigen cross-presentation was measured in the ovarian tumor environment using the model antigen OVA [79]. Ovarian tumor-associated antigen presenting cells phagocytose antigens in the ovarian tumor environment, but these immune suppressed cells poorly cross-present antigen to prime CD8⁺ T cell responses [65, 70, 75]. Following injection of chicken ovalbumin into the tumor environment, *cps* treatment rapidly reversed immune suppression within 18 hours and activated the ability of antigen presenting cells to present the OVA peptide (OT-1) by MHC-I molecules to re-activate the priming of CD8⁺ T cell responses.

Treatment with *cps* markedly increased cellular recruitment to the ovarian tumor and the spleen [79]. The number of splenocytes increased following *cps* treatment reflected by significant increases in the number of CD45⁺CD11c⁺ cells, macrophages, B cells, NK cells, CD4⁺ T cells and CD8⁺ T cells. Cellular recruitment was also enhanced at the tumor site. The number of CD45⁺, CD45⁺CD11c⁺, CD8⁺ T cells, and CD4⁺ T cells were increased at

the peritoneal tumor location, and the percentage of CD45⁺CD11c⁺ and CD8⁺ T cells was also increased in the tumor environment. Conversely, the percentage of Foxp3⁺CD4⁺ T-regulatory cells (Tregs) was decreased in the ovarian tumor environment by *cps* treatment [79]. This observation is consistent with several reports showing that *Toxoplasma* infection significantly suppresses the development of Foxp3⁺CD4⁺ Treg populations [84, 85]. Importantly, higher levels of Foxp3⁺CD4⁺ Tregs have been shown to represent an adverse prognostic factor in human ovarian cancer [86, 87]. No changes were found in TH17 cells by *cps* treatment, and mice that lack TH17 cells (IL17a knockout mice) responded as well to *cps* therapy as normal mice [79].

These observations suggested a global reversal of tumor-associated immune suppression by *cps* treatment activated immunity to tumors [57, 79] (Figure 1). Immune activation by *cps* treatment could prime new antitumor CD8⁺ T cell responses or could potentially strengthen existing CD8⁺ T cell responses that were being suppressed in the ovarian tumor environment. The *ex vivo* *cps* treatment of immune suppressed cells in the ovarian tumor environment increased expression of the CD69 activation marker on T cells [79]. The number of splenic CD8⁺ T cells that expressed central memory markers (CD44⁺CD62L⁺) was increased by *cps* treatment. Ovarian antigen-specific CD8⁺ T cell responses in the spleen as well as in the tumor were increased by *cps* treatment [79]. Correspondingly, the number of activated CD8⁺ T cells that expressed cytolytic granzyme B in response to tumor antigens was also markedly increased by *cps* treatment. We performed adoptive transfer studies to determine whether *cps* elicited ovarian tumor antigen-specific CD8⁺ T cell responses. While T cells obtained from untreated tumor-bearing mice lacked the ability to control solid ovarian tumors, T cells obtained from *cps*-treated mice exhibited a potent ability to suppress ovarian tumor development [79]. Outstanding questions for future research are listed in Box 1.

Box 1

Outstanding questions

- Do parasite molecules (profilin, STAg) that induce IL-12 via interaction with TLR play any role? Why is MyD88-independent IL-12 production sufficient? What is the source of MyD88-independent IL-12 production?
- Is active invasion and secretion of parasite molecules required for stimulation of the antitumor response? If secretion of parasite molecules is required, do these molecules trigger host cell responses by interaction with extracellular or intracellular receptors? Do secreted parasite molecules actively manipulate the host cell and tumor microenvironment?
- Which innate cytokine and chemokine responses are necessary for activation of antitumor responses?
- Are cell types newly recruited to the tumor microenvironment important? Are myeloid derived suppressor cell types disarmed? Which cell types contribute the most important antitumor mechanisms?
- How does *cps* activate antitumor CD8⁺ T cell responses? Can *cps* stimulate CD8⁺ T cell immune memory to prevent future tumor recurrences? Can *cps* be engineered as a vaccine to generate specifically targeted CD8⁺ T cell antitumor responses?
- Will *cps* stimulate strong antitumor responses in humans?

Concluding remarks

The application of *cps* as an immunotherapeutic cancer vaccine for treating established solid tumors shows great potential. The tractable *ku80* genetic model that now permits efficient targeted genetic manipulation in *Toxoplasma* [88, 89] has enabled the recent development of improved genetically defined, nonreverting *cps* strains [88, 90]. The powerful but well-controlled innate immune responses elicited by *cps* stimulate remarkable immunotherapeutic antitumor responses, and consequently, it is necessary to understand the mechanisms used by *cps* to trigger these beneficial responses in immune suppressed tumor environments. Addressing this future challenge will involve genetics, cell biology, and immunology, and the reward of this endeavor could be the development of immunotherapeutic cancer vaccines based on engineered *Toxoplasma* uracil auxotrophs that have the ability to effectively regress established tumors and to then prevent their recurrence.

Acknowledgments

We apologize for not citing the valuable studies of many colleagues owing to space limitations. Research in D.J.B.'s laboratory is supported by the National Institutes of Health (R01 AI041930, R21 AI104514).

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Highlights

- Immunotherapy based on avirulent *Toxoplasma* reverses tumor-associated immune suppression
- *Toxoplasma* activates tumor-specific CD8⁺ T cell responses.
- *Toxoplasma* immunotherapy regresses established solid tumors.

Figure 1.

Overview of how *cps* reverses tumor associated immune suppression and activates immunity to tumors. Tumors create highly immunosuppressive microenvironments (left panel). High levels of IL-4 and IL-10 promote maintenance of M2 macrophages and myeloid derived suppressor cells (MDSC) [18, 19]. Dendritic cells (DC) do not produce IL-12 [20, 21]. Vascular endothelial growth factor (VEGF) promotes angiogenesis [17, 65]. T-regulatory (Treg) cells suppress the functions of CD8⁺ T cells [9, 10]. Immune recognition of tumor cells is suppressed and potential effector mechanisms that could act to kill tumor cells are being suppressed at multiple levels [9–14]. *Cps* treatment stimulates innate and adaptive immune responses that reverse immunosuppression and activate immunity to tumors (right panel). Production of IL-12 [79] from activated cell types such as DCs promotes the M1 macrophage phenotype and suppresses the role of MDSCs. Treg suppressor cell populations are reduced [79], allowing the emergence of antitumor CD8⁺ T cells. Natural killer (NK) and other cell types are recruited [57, 79]. Interferon- γ is produced in the tumor microenvironment [57, 79]. Activated macrophages, CD8⁺ T cells, NK cells, and inflammatory cytokines contribute mechanisms that result in tumor cell killing, causing tumor regression [57, 79].

Table 1Tumor treatment studies using the *cps* strain

Tumor	Treatment	Site	Refs
B16F10	<i>cps</i> (1.5×10^7)	Intradermal	[57]
Lewis lung	<i>cps</i> (1.5×10^7)	Intradermal	[57]
BRAF/PTEN	<i>cps</i> (1.5×10^7)	Intradermal	[57]
UPK10	<i>cps</i> (1.5×10^7)	Intradermal	[57]
ID8- <i>Vegf-A</i>	<i>cps</i> (2.0×10^6)	Intraperitoneal	[79]
ID8- <i>Vegf-A</i> (immune)	<i>cps</i> (2.0×10^6)	Intraperitoneal	[79]
ID8- <i>Defb29/Vegf-A</i>	<i>cps</i> (2.0×10^6)	Intraperitoneal	[79]
ID8- <i>Defb29/Vegf-A</i>	Adoptive T cells	Intradermal	[79]