

Boosting effect of pre-existing immunity on anti-cancer immunotherapies

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Abstract

Intratumoral application of intact microbes and/or foreign antigens is a promising strategy in anti-cancer immunotherapy since, after the induced immune activation, antigen spread toward tumor-associated antigens and/or neo-antigens can generate an anti-tumor vaccine effect. However, the strong immunosuppressive nature of the tumor microenvironment (TME) has been shown most often to curb the mounting of an effective immune response. By examining the literature, we found evidence that pre-existing immunity to foreign antigens has a crucial role in determining the actual degree of immune activation obtainable. Therefore, assessing or even voluntarily inducing pre-existing immunity to specific foreign antigens can help develop successful immunotherapy. Furthermore, pre-existing immunity to common pathogens developed by previous infection and/or vaccination could be exploited to turn cold tumors into hot ones, a most desired condition for successful checkpoint inhibitor based anti-cancer immunotherapy.

Introduction

Tumors are complex ecosystems in which neoplastic cells, resident mesenchymal cells, endothelial cells, infiltrated inflammatory immune cells and even eventual pathogens and commensal entities (such as viruses, bacteria, mycoplasma and fungi) live in an altered microenvironment, the tumor microenvironment (TME) [1,2].

Immune cells, in particular, influence tumors at all stages of disease, from early neoplastic transformation to metastatic dissemination [3]. The immune system, which is able to eliminate cancerous cells at an early stage (in the immune surveillance phase), must obviously lose its ability to inhibit tumor formation for their clinical appearance to manifest. Tumors pass through immunoediting mechanisms during their progression and find ways to grow without immune control restriction, in the end (immuno-evasion) [4]. In addition, biochemical and metabolic features of established tumors, such as hypoxia and elevated levels of lactate, actively contribute to immunosuppression by directly inhibiting effector T cell functions [5] and also special enzymatic activities in tumors, like indoleamine 2,3-dioxygenase (IDO), can suppress effector T cell activation, promote generation of Tregs and infiltration of myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) suppressing the antitumor response [6,7]. The immune system has a crucial role in therapy and its anticancer activity must be re-awakened for patients to achieve significant remissions. In fact, they would not likely be cured by chemotherapy, radiotherapy or surgery alone, but by their own immune system killing residual tumor cells [8].

In the last years, we are learning how to play with the immune system in the fight against cancer [9] and removing some of the molecular brakes to T cells with checkpoint inhibitors (CIs) has been shown as extremely helpful in various types of malignancy. Patients with immunologically “hot” tumors, characterized by functional antigen presenting cells (APCs) and/or reactive tumor infiltrating lymphocytes (TILs) usually respond well to these drugs [10,11]. On the contrary, patients bearing “cold” tumors, characterised by either “immune deserts” or the presence

of regulatory cells (as Tregs, Bregs, MDSCs, and TAMs), are much less responsive [12]. Therefore, strategies to recruit reactive immune cells and/or revert immune tolerance mechanisms into the TME are the next frontier under intensive exploration in cancer immunotherapy [13-15].

Among strategies that aim at “turning tumors hot”, systemic chemotherapy is commonly employed in the clinics under the rationale that it could induce immunogenic cancer cell death and the release of cancer antigens, prompting the immune response. This strategy, however, appears inherently flawed since standard chemotherapy regimens are so strongly immunotoxic that patients, left by chemotherapy with an impaired immune system, are often unable to benefit from immunotherapy [16,17]. Nonetheless, a few chemotherapeutic agents, especially when employed at doses much lower than those usually prescribed, have shown immunomodulatory effects [18]. Physical ablative techniques, radiotherapy in particular, have shown to cause immunogenic cancer cell death with lesser debilitating effects on the immune system. Therefore, their potential synergy with CIs immunotherapy is particularly promising [19-22].

Among many other possible strategies, locally applied microbial agents, such as whole pathogens, pathogen associated molecular pattern agonists (PAMPs), microbial peptides and m-RNA vaccines would appear particularly suited to prompt an immune attack against cancer [23]. This strategy is inspired both by a basic tenet of immunology affirming that the immune response against foreign antigens is strong [24] and by the possibility that strong immune activation could lead to antigen spread toward tumor associated- (TAA) and tumor neo-

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antigens (TNAs) [25,26]. Exposure to foreign antigens usually elicit a local immune response in healthy tissues and successive antigen exposures can boost the immune response. In fact, the so-called booster dose is well employed in vaccination protocols. Antigen spread toward self-antigens can sometimes take place and give rise to undesired autoimmune reactions. Similarly, allowing each patient's immune system to select accessible antigens in situ and generate an antigen agnostic tumor vaccine effect [27-31].

Researchers are actively working to improve the immunological potency of those immunotherapeutic agents in various ways; by using novel adjuvants, adding immunostimulatory biomolecules (GM-CSF, IL-2, IL-12, etc.), employing novel slow-release immunomodulating matrices, etc. [32]. In this work we have collected the evidence suggesting that a well-known immunological mechanism used in classical vaccines, i.e. the booster effect of pre-existing immunisation, can substantially increase the anticancer potential of various immunotherapies.

Microbial based cancer immunotherapies

The immune system's ability to kill pathogen infected cells could be exploited, in principle, by infecting cancer cells and forcing them to expose foreign epitopes to the immune system [33]. Historically, this kind of immunotherapy has been pioneered by William Coley, more than a century ago when, after having observed spontaneous tumor regressions following infections in cases of soft tissue sarcoma, he proceeded to cause such infections on purpose by locally injecting exposed tumor lesions with a bacterially-derived preparation (Coley's toxins) [34]. Nowadays, more refined preparations are available, such as recombinant viruses, bacteria, fungi, and microbial derived molecules that specifically activate defined pathogen associated molecular patterns (PAMPs). These agents are usually delivered intratumorally in order to bypass systemic immune recognition and neutralisation [13].

An exhaustive list of microbial based anticancer agents is outside the scope of this paper. However, a paradigmatic example is that of recombinant viruses, engineered to selectively infect and kill malignant cells. First generation of these vectors were based on adenoviruses and showed insufficient activity in clinical trials [35]. These trials, however, helped in understanding that a substantial part of their anti-tumor activity was not due to their cell killing ability but to the concomitant activation of the immune system recognising infected cells [35-37]. The idea that tumor cell lysis by the pathogen was essential for anticancer activity was further discredited by the evidence that an inactivated oncolytic virus was capable of stimulating antitumor immunity better than its active oncolytic analog [38].

Currently developed viral agents are designed to specifically boost immune response and this has brought considerable improvements, to the point that T-vec (talimogene), an herpes virus-1 modified oncolytic virus encoding granulocyte macrophage-colony stimulating factor (GM-CSF), gained Food and Drug Administration approval for the treatment of melanoma. Other vectors, based on vaccinia viruses encoding GM-CSF are currently under clinical testing [39] and the current state of the art indicate a strong synergy with systemic CIs immunotherapy [40].

The idea that anticancer activity of these viral vectors is immune-mediated prompted researchers to investigate potential effectiveness of other, easier to obtain commercial attenuated viral strains, as anticancer agents for intratumoral immunotherapy. The advantage of

repurposing marketed agents is that their clinical development would be much simplified based on their already known safety records [41].

The role of pre-existing immunity

The role of pre-existing immunity in boosting the immune-mediated antitumor activity of microbial based agents has been notably addressed in the studies reported in Table 1.

The role of pre-existing immunity to oncolytic viral vectors, in particular, has been specifically studied by Ricca et al., using the Newcastle Disease Virus (NDV). They reported that, despite pre-existing immunity to NDV limits its replication in tumors, tumor clearance, abscopal effects and mice survival were superior in NDV-immunized mice [42].

Similar observations were made with Oncolytic Vaccinia Viruses delivered in murine tumor models. Lymphocyte infiltration was shown to increase in pre-immunized mice compared to naïve ones [43]. An effective priming effect of previous systemic exposure, preceding virus

Table 1. Studies addressing the role of pre-existing immunity in boosting immune activating capability of ITD foreign antigens

Ref. N	Title	Agent
41	Repurposing rotavirus vaccines for intratumoral immunotherapy can overcome resistance to immune checkpoint blockade	inactivated Rotavirus
42	Pre-existing Immunity to Oncolytic Virus Potentiates Its Immunotherapeutic Efficacy	Newcastle disease virus (NDV)
43	Redirecting adaptive immunity against foreign antigens to tumors for cancer therapy	LacZ expressing adenovector (Ad/CMV-LacZ) Vaccinia virus
44	Intratumoral Vaccination and Diversified Subcutaneous/ Intratumoral Vaccination with Recombinant Poxviruses Encoding a Tumor Antigen and Multiple Costimulatory Molecules	Avipox vector (rF-CEA/TRICOM)
45	Exploiting pre-existing immunity to enhance oncolytic cancer immunotherapy	Adenovirus coated with tumor-specific peptides and Tetanus or Diphtheria or Pertussis antigens (TT-OVA-PeptiCRAd)
48	Repurposing the yellow fever vaccine for intratumoral immunotherapy	inactivated Yellow Fever strain 17D
49	Virus-specific memory T cells populate tumors and can be repurposed for tumor immunotherapy	viral peptides
50	CRM197 and cancer: effects of intratumoral administration	CRM197 protein
51	Recurrent Glioblastoma Treated with Recombinant Poliovirus	inactivated recombinant Poliovirus
52	Combined Systemic and Intratumoral Administration of Human Papillomavirus Vaccine to Treat Multiple Cutaneous Basaloid Squamous Cell Carcinomas	Gardasil 9™ HPV peptide vaccine
53	Systemic and intratumoral 9-valent human papillomavirus vaccine treatment for squamous cell carcinoma in situ in a renal transplant recipient	Gardasil 9™ HPV peptide vaccine
57	Intratumoral delivery of an HPV vaccine elicits a broad anti-tumor immune response that translates into a potent anti-tumor effect in a preclinical murine HPV model	HPV E7 epitope plus poly(I:C)
67	Intratumoral injection of the seasonal flu shot converts immunologically cold tumors to hot and serves as an immunotherapy for cancer	inactivated Human Influenza vaccine

ITD, was also observed with an Avipox Vector expressing a TAA together with multiple costimulatory molecules [44]. More recently, pre-existing immunity was exploited to enhance immunotherapy with a vector named TT-OVA-PeptiCRAd, which contains tetanus antigens together with TAAs [45]. In this case, pre-immunization against tetanus was exploited in order to enhance the cancer treatment. In fact, when animals pre-immunized with an ordinary vaccine to tetanus were then treated with the new hybrid viral vector carrying the same immunogen, a dramatic improvement in tumor-specific immune response was obtained. Cooperation of CD4+ and CD8+ T cells was shown to be responsible of the anti-tumor immunity, as it was expected from recent studies on the interplay between lymphocyte populations [46]. Involvement of CD4+ T cells, in particular, provided signals that improved functionality of CD8+ T cells within the TME [47] as their depletion prior to tumor challenge resulted in complete loss of antitumor effects. Interestingly, in non-vaccinated mice, the superiority of the TT-OVA-PeptiCRAd was lost, highlighting the prerequisite of pre-existing immunity in order to exploit CD4+ T cell memory boosting effect.

Using different pathogen antigens (from Diphtheria and Pertussis) similar infiltration of effector memory CD4+ T cells in the TME was observed that correlated with CD8+ T antigen-specific TILs and the level of tumor growth control [45]. The mechanism, thus, was not restricted to tetanus antigens and is in principle applicable to a variety of other vaccine formulations.

The role of pre-existing immunity has also been scrutinized in experiments with an attenuated strain of Rotavirus, given intratumorally to mouse bearing syngeneic transplantable tumors. Given intratumorally, viruses were able to synergize with and overcome resistance to anti-CTLA-4 or anti-PD-1 L1 CIs in refractory tumor models. According to these authors, pre-vaccination to those virus strains did not spoil their strong anticancer effect [41].

Pre-vaccination potentiated the growth inhibitory effect of syngeneic tumors in mice of an intratumorally inoculated attenuated yellow fever virus strain (17D). Tumor progression was delayed in a manner mediated by CD8 T cell immunity. Measurable effects were recorded against non-injected concomitant tumor lesions, clearly indicating that viral ITD generated an immune-mediated abscopal effect. Synergy with systemic immunostimulatory anti-PD1 or anti-CD137 antibodies was boosted by systemic pre-vaccination with the same viral vector [48].

The role of pre-existing immunity to specific viral peptides has been studied by Rosato et al. They showed that ITD of peptides, which can mimic viral reinfection at the level of tumors, can restore susceptibility of resistant tumors to CIs immunotherapy [49].

We argue that similar effects could be obtained with other peptides. In fact, in an old phase I/II trial, even bacterial proteins commonly used in vaccine preparations both as a carrier of glucidic epitopes and delivered to cancer patients by ITD could induce a response against a variety of solid tumors, as a function of pre-existing immunity (measured by antibody titer and delayed type hypersensitivity) [50].

The boosting effect of pre-immunisation has been exploited recently in a clinical trial with a genetically engineered Poliovirus, delivered intratumorally after pre-vaccination with a classical polio vaccine, in patients affected by recurrent glioblastoma. In this trial, the measured anticancer effect was stronger in those patients who had achieved higher anti-poliovirus serological titers before poliovirus ITD [51].

In two recently published case reports, a recombinant anti-papillomavirus vaccine, composed by viral peptides, (Gardasil 9™) was employed against cutaneous malignancies with astounding results. The therapy consisted of pre-vaccination by intramuscular doses (as in the standard vaccine protocol) and later repeated monthly vaccine injection into tumor lesions. Not only those injected, but all other lesions showed complete regression and response was durable (at least in the 18-month period of follow-up observation) [52,53].

Since pathogen-specific (cytomegalovirus, influenza virus, Epstein-Barr virus, etc.) CD8+ T cells have been observed to actually infiltrate mouse and human tumors [49,54-56], we argue that anti-pathogen matching vaccines could be exploited as agents to improve anticancer immunotherapy. In fact, specifically reactive immune cells could be stimulated by ITD of matching vaccine preparations. The selection of a proper vaccine could be easily performed by measuring pre-existing immunity with simple serological testing.

The role of Innate Immunity

In examining the role of pre-existing immunity to foreign antigens for anticancer immunotherapy, the role of the innate arm of immunity should be considered. The literature records indicate that innate immunity activation within the tumor microenvironment can substantially improve epitope spreading of adaptive immunity, facilitating and broadening the immune response to TAAs and TNAs. This was demonstrated in a murine syngeneic tumor model, for instance, when using the E7 papillomavirus peptide together with a TLR3 agonist, the poly(I:C). The combined formulation provided an enhanced and durable tumor-specific response, particularly in previously vaccinated mice. The poly(I:C)-induced activation of innate immunity lead to coordinated upregulation of chemokines and integrins which facilitated T cell trafficking to the tumor site [57].

Today, a long list of toll-like receptor agonists is under study for immuno-oncological Purposes [58]. Among them, toll-like receptor 3 (TLR3) agonist poly ICLC (Hiltonol), which showed efficacy in transplantable mouse tumors and synergy with checkpoint inhibitors [59,60] and is now in human trials (NCT03721679) m-RNA encoding TLR 4 agonist (together with CD70 and CD40 stimulatory ligands) is investigated in patients in early stage breast cancer (NCT03788083); G100 synthetic TLR4 agonist is evaluated in Merkel cell carcinoma and soft tissue sarcoma, in combination with radiotherapy. (NCT 02501473) Synthetic agonists of TLR 7/8 receptors, such as imiquimod and resiquimod, developed as topical treatments for basal cell carcinoma, melanoma, skin neoplasms and even common warts; and imiquimod as treatment for breast cancer in combination with radiotherapy [61]. TLR7/8 agonist NKTR-262 is also currently being studied in patients with locally advanced or metastatic solid tumors (NCT03435640) since preliminary results from the phase I/II trials showed encouraging results in controlling various types of oncologic diseases [61,62].

Synthetic endosomal TLR9 agonists CpG oligonucleotides (CpG-ODN) have been successfully employed against human lymphoma, showing some sign of tumor regressions paralleled by changes in the tumor lymphocytic infiltrates [61] and in combination with low dose limited field radiotherapy, their anti-lymphoma activity is potentiated [63]. In a phase II study utilizing perioperative local administration into the resection cavity in patients with newly diagnosed glioblastoma, followed by standard of care therapy following resection, survival benefits have not been observed [64].

Notably, the combination of the TLR9 innate immunity stimulant with an OX40 agonistic antibody has been shown to be able to

generate complete tumor remissions in mice models [65] and further combination with radiotherapy is under evaluation in two clinical trials (NCT03410901, NCT03831295). Activity of the combination depends both on immune activation mediated by the TLR9 agonist and the boosting effect of the OX40 agonist on resident TILs [65]. Dose, timing of delivery and precise histological localisation of these agents can have crucial influence on their activity [66].

The role of formulation

Despite an exhaustive discussion on the role of vaccine formulation is outside the scope of this paper, we provide a few paradigmatic examples below, showing how reasoning only on the role of antigens is a misleading process. In fact, the role of adjuvants, co-formulants and the matrix are of paramount importance in determining the effects of a vaccine.

Adjuvants, for instance, can determine unexpected results when foreign antigen vaccines are delivered intratumorally. In fact, in a study on the anticancer properties of flu vaccines, Newman et al. observed that only unadjuvanted formulations were effective against tumors whereas adjuvanted ones were ineffective. This was most probably due to a detrimental attractive effect of adjuvants, when the vaccine was injected intratumorally, on immunosuppressive regulatory B cells within the TME [67].

A detrimental effect of the adjuvant, was observed by Hailemichael et al., when investigating the lack of synergy with CTLA-4 blockade exhibited by the gp100 tumor antigen vaccine subcutaneously injected with the Freund's adjuvant in melanoma patients. This time, trafficking of activated T cells to the tumor site was impaired by a persistent attraction effect exerted by the adjuvant at the extra-tumoral injection site. The same gp100 peptide vaccine was effective, instead, when administered subcutaneously again but dissolved in phosphate buffer saline [68].

The above mentioned examples illustrate how controlling immune cell dynamics is therefore a crucial aspect for effective immunotherapies. The concept has been paradigmatically exploited in novel vaccines matrices engineered to persistently attract immune processing cells to TAAs and/or TNAs. The polyethylene glycol-alginate cross-linked matrix carrying tumor antigens plus GM-CSF and a CpG oligodeoxynucleotide (CpG-ODN), implanted subcutaneously in tumor bearing mice, strongly attracted dendritic cells mediating the development of effective antitumor immunity [69]. With a different strategy, an extracellular matrix-binding IL-12 chimeric molecule was delivered intratumorally to persistently recruit T-cells toward the tumor lesions, leading to immune eradication of established malignancies and clear abscopal effect [70].

Perspectives and Challenges

Pre-immunisation against foreign antigens can be exploited to boost the efficacy of various microbial-based cancer immunotherapies, as it happens with the "booster dose" is commonly used in common vaccine protocols. The booster effect can take place even when the injection is performed intratumorally. Development of immunity first outside the TME, however, should be required to by-pass immune tolerance mechanisms that are in place inside the TME.

Immune stimulation with foreign antigens can be harnessed both by using foreign antigens to indirectly attract antigen processing cells toward purified TAAs, TNAs and/or tissue lysate cancer vaccines and to deliver foreign antigens directly into tumor lesions, after having

checked available pre-existing immunity or having developed it by vaccination. Foreign antigen preparations and existing vaccines could become interesting candidates, readily available thanks to their well-known safety profiles, as anticancer immune modulating/stimulating agents for ITD. Pragmatically, the main difficulty in planning similar interventions, is the involvement of interventional radiologists, oncologists and laparoscopic surgeons in clinical trials. In fact, despite the right tools to reach almost every district in the body and inject tumor lesions with great precision and efficacy are available, these professionals are still hard to find except at very few excellence centers worldwide [71].

Compliance with Ethical Standards

M.Conti declares that he has no conflict of interest. This article does not contain any studies with human participants or animals performed by the author.

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