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Trends in Cancer

Forum

Intratumoral immune cell manipulations as a strategy to enhance cancer vaccine efficiency

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Shortcomings in cancer vaccine development are attributable to weak and transient anti-tumor cellular responses in the tumor microenvironment. This restriction of efficacy may be due to an intratumoral immunosuppressive milieu, consisting of regulatory T cells, M2 macrophages, and myeloid derived suppressor cells. Here, we analyze recent advances and propose future directions in the modulation of cellular state propensities combined with cancer vaccines.

Introduction

Cancer immunotherapies such as checkpoint inhibitors, chimeric antigen receptor T cells, and cancer vaccines have been used in clinical practice. Among them, cancer vaccines have made the least impact on cancer treatment, with only two drugs, sipuleucel-T (Provenge) and T-VEC, receiving FDA approval; these have experienced limited uptake in the market due to unimpressive cost–benefit ratios. Cancer vaccine developments have repeatedly failed to deliver clinically significant results for several reasons, including difficulties in delivery and uptake of drugs and systemic side effects. Above all, lack of sustained immunogenicity is a major hurdle for successful cancer vaccine development.

Tumor microenvironments (TMEs) of solid tumors are often highly immunosuppressive, with reduced innate and adaptive immune responses to cancer cells that lead to a self-perpetuating immunosuppressive cycle [1]. Recent evidence suggests that manipulating the intratumoral phenotypes of two key immune cells, macrophages and T cells, could lead to significant increases in the scale and duration of adaptive immune responses. Broadly speaking, macrophages and T cells within the TME can be divided into antitumorigenic M1 and $CD8^+$ T cells and tumorigenic M2 and T regulatory (Treg) cells. Here, we discuss methods to augment TME polarization. We propose that deploying adjuvants to modify phenotypes within the TME could overcome current challenges in cancer vaccine use, leading to the development of efficacious combination therapies.

Modulation of M1 and M2 macrophage ratio

Macrophages within the TME can be simplistically divided into two classes: antitumorigenic M1 cells characterized by the release of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-2, and IL-6, which result in desirable phenotypes for antitumor immune responses, and tumorigenic M2 cells characterized by secretion of immune-dampening cytokines such as IL-10 and transforming growth factor (TGF)-β, leading to undesirable immune outcomes. Unlike other cells, the phenotypic state of macrophages is relatively plastic [2] and short-term influence by local TME stimuli can have a marked effect on phenotype proportions. Therefore, recent immunological work has been aimed at developing cancer vaccines and vaccine adjuvants that can promote the antitumorigenic M1 phenotype and disrupt the self-reinforcing tumorigenic and immunosuppressive effects of the TME.

Decorated nanoparticles can serve as a suitable platform for delivering targeted and controlled immune stimulation over an extended period. Mannose- and hyaluronic-acid- decorated iron nanoparticles improved M1:M2 TME ratios, $CD8^+$ T cell recruitment and tumor volume reductions in a TC-1-cell-induced, syngeneic murine tumor model [3]. Mannose improves M2-specific cell targeting (via CD206) whilst hyaluronic acid stimulates M1 polarization. Other iron-based vaccine adjuvants produce immunogenic necroptosis of TC1 cells, triggering DAMP release and boosting MHC-1 antigen cross-presentation on dendritic cells and $CD8^+$ infiltration into the TME (Figure 1) [4]. These markers of successful immune activation correlated with reduced tumor size and increased survival in a 4T1 breast cancer tumor model in BALB/c mice [4]. These studies indicate that the delivery scaffolds supporting cancer vaccines could improve antitumorigenic responses independently of antigenic vaccine components.

Moreover, these immunogenic responses appear to be robust. In a B78 melanoma mouse model [2] the ratios of nitric oxide synthase (NOS)2: arginase (ARG)1, TNF-α: TGF-β), and IL-1β: IL-10 were all significantly increased when using PIC (polylysine, iron oxide and CpG) nanoparticles with radiotherapy compared to radiotherapy alone. These findings indicate that nanoparticle-based vaccine constructs can enhance antitumorigenic M1 macrophage populations beyond inflammatory damage induced by conventional therapy. PIC administration showed nanoparticle accumulation 5 days post-administration and proinflammatory STING signaling 15 days after administration [2], indicative of persistent antitumorigenic changes.

These phenotype-modifying vaccine designs have resulted in sustained, systemic immune responses with an initial course of vaccination also leading to antitumorigenic responses at mock-metastatic sites in multiple murine cancer models [2–5]. These data

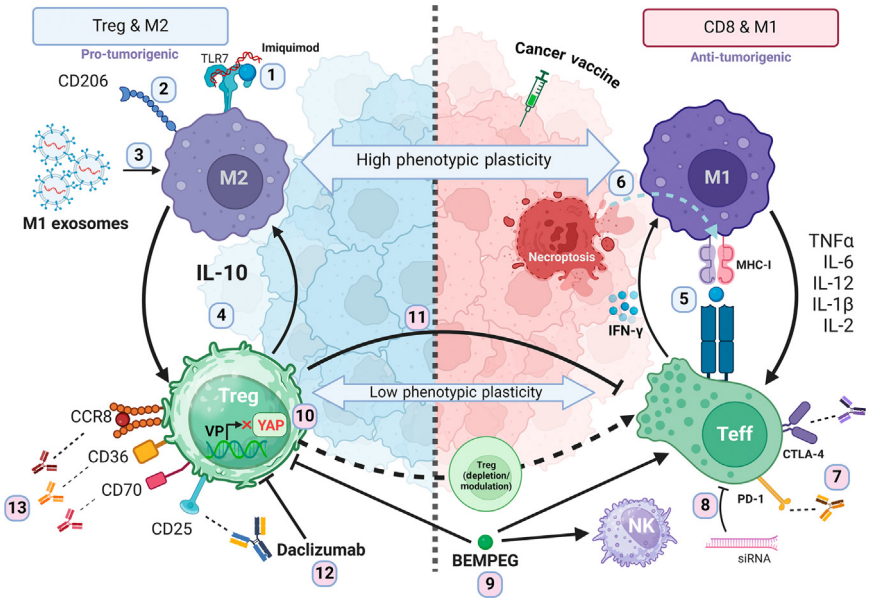

Figure 1. T cells and macrophages interactions and modes of manipulation of phenotypic plasticity in the tumor microenvironment to enhance therapeutic cancer vaccine efficacy. (1) TLR-7/8 drug agonists (e.g., imiquimod) promote conversion of M2 to M1 phenotype by stimulating production of proinflammatory type-I IFNs. TLR7 stimulation activates MyD88 adaptor protein, linking TLR to IRAK-1. Downstream effects of the subsequent phosphorylation pathway cause activation of IRF, promoting proinflammatory secretions, e.g., IFN-γ/α/β. (2) Characteristic markers for M2 macrophages include CD206 which act as innate receptors for bacteria and mannose. Secretions are characteristically anti-inflammatory cytokines including IL-10, IL-13, Il-14, PPAR-γ, VEGF, CCL-17, and CCL-22. (3) Exosomes derived from M1-polarized, proinflammatory macrophages as an immunopotentiator for a cancer vaccine. The M1 exosomes induced the release of a pool of Th1 cytokines and induced a stronger antigen-specific cytotoxic T cell response. (4) The immunosuppressive TME can self-perpetuate wherein the Treg cells and M2 macrophages are involved in a positive feedback loop from interactions including the secretion of anti-inflammatory IL-10. (5) Interaction of M1 macrophages with Teff cells in the TME: proinflammatory cytokines secreted from activated effector T cells (TNF-α, GM-CSF, and IFN-γ) induce activation of M1 phenotype macrophages, following recognition of a presented antigen. IL-12 is secreted from activated macrophages, stimulating $CD8^+$ activity in a positive feedback loop. Increased M1 activation therefore can lead to elevated Teff cell action, inducing tumor regression. (6) Iron-based vaccine adjuvants have been shown to induce necroptosis within tumor cells. This immunogenic cell death modality leads to improved MHC-I antigen cross-presentation and tumoral $CD8^+$ Teff cell infiltration. (7) A combination of anti-CTLA-4 and anti-PD-1 led to activation and proliferation of cancer neoantigen-vaccine specific T cells, as well as a decrease in tumor-infiltrating Treg cells. (8) siRNA can be used as an adjunct to limit PD-1 expression, preventing the $CD8^+$ Teff cells recognizing the cancer cells as self. (9) Bempegaldesleukin (BEMPEG: NKTR-214), an engineered IL-2 cytokine prodrug was designed to rapidly expand $CD8^+$ T and NK cells and Teff-cell-derived IFN-γ and TNF-α selectively depletes intratumoral Treg cells. (10) YAP is a transcriptional coactivator and is vital for Treg cell function. Inhibiting the YAP/activin/SMAD axis by using VP in Treg cells synergistically enhanced the antitumor efficacy of GM-Vac (lethally irradiated B16 cells producing GM-CSF) vaccine when combined. (11) Treg cells suppress Teff cells via many mediators such as CD36, CD70 and CCR8. (12) CD25-blocking monoclonal antibody, daclizumab, resulted in a robust peptide-specific $CD8^+$ T cell responses to an experimental cancer vaccine. Daclizumab acted on a subset of Treg cells via cytokine deprivation. (13) Antibody against the markers preferentially expressed by the Treg cells such as CD36, CD70, and CCR8 can be used as a target for Treg cell depletion. (14) Intratumoral vaccine delivery produces localized immune stimulation, helping to overcome two challenges of cancer vaccine development: targeted delivery following systemic administration and adequate dosing via systemic administration. Abbreviations: CCL, chemokine CC ligand; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IRAK-1, IL-1-receptor-associated kinase; IRF, IFN regulatory factor; NK, natural killer; PD-1, programmed death 1; PPAR-γ, peroxisome proliferator-activated receptor-γ; Teff cell, T effector cell; TLR, Toll-like receptor; TME, tumor microenvironment; TNF, tumor necrosis factor; Treg cell, T regulatory cell; VEGF, vascular endothelial growth factor; VP, verteporfin; YAP, Yes-associated protein.

indicate that successful cancer vaccine implementation could reduce future dissemination. Human studies should be conducted to determine if incorporating such adjuvants into cancer vaccines could decrease the likelihood of recurrence or metastasis.

Although the crucial roles of $CD8^+$ T cells are well defined, there are mixed results regarding the role of $CD4^+$ T cells in the cancer vaccine efficacy. A synthetic long peptide vaccine targeting MHC-I- and MHC-II-restricted neoantigen shows an improved overall survival in a Panc02-induced tumor compared to MHC-I targeted peptides alone (8/15 mice vs 3/15 mice) [6]. Most notably, the combined MHC-I- and MHC-II-targeted vaccine demonstrated increased myeloid cell infiltration with a significantly increased intratumoral M1:M2 ratio compared to the solely MHC-I-targeted, vaccine-treated groups. This highlights an essential effect of $CD4^+$ T cells on macrophage polarization balance, which is likely explained by the interferon (IFN)-γ signaling observed from $CD4^+$-stimulated populations. This finding also demonstrates the potential impact of intelligent vaccine design on disease outcomes.

Other technical aspects of tumor vaccine delivery can also be addressed using nanoparticle-based construct methods. For example, appropriate vaccine targeting is needed to avoid systemic side effects. Surface coating with mannose effectively targeted nanoparticle to CD206, characteristic of M2, leading to a twofold uptake of the modified nanoparticle into M2 macrophages relative to the base nanoparticle (ferumoxytol) [3]. Similarly, lipid-coated calcium phosphate nanoparticles have been used as peptide vaccine carriers alongside co-delivery of M1 macrophage exosomes. The M1 exosomes led to significant tumor growth reduction in B16F10 melanoma mouse models compared to vaccine alone [7]. These findings reinforce two ideas: (i) M1 cellular components (here exosomes)

can propagate the proinflammatory environment to new sites of immunologic activity; and (ii) vaccine components can be combined to target phenotypic modification in specific cell lines.

Although some cancers permit intratumoral administration, deep or disseminated cancers can make targeted deployment of cancer vaccines more challenging. Advances in targeted phenotypic activation with TMEs can be seen in photoactivating vaccine delivery constructs designed utilizing photothermal response PTEQ polymers. These have been administered in combination with programmed death ligand (PDL)-1 siRNA, permitting temporal and spatial control over treatment, which reduces systemic side effects [5]. Photoactivation yielded modest improvements in M1:M2 ratio within the TME compared to the construct alone (PTEQ/siPDL1 construct plus laser activation saw changes of M1 from 2% to 23%, and M2 from 6% to 2% compared to PBS). In a CT26 murine colorectal carcinoma model, 100% of mice pretreated with whole tumor cells plus PTEQ/siPD-L1 polymer constructs achieved eradication of CT26 cells when rechallenged 14 days later, compared to 0% of mice when pretreated with PBS, demonstrating a strong and sustained induction of adaptive immune responses [5].

This evidence demonstrates that an overall modulation in macrophage phenotype from the tumorigenic M2 to the antitumorigenic M1 type yields improved cancer vaccine response. Individual vaccine components can provide properties that have hitherto hampered cancer vaccine development, namely targeted delivery and sustained immunogenic responses. The vaccine components could be recombined and repurposed to tailor efficacy to a wide range of cancers and disease stages with the potential to drastically expand a practicing clinician's toolbox (see Clinician's corner).

Modulation of Treg–T effector cell ratio

Therapeutic cancer vaccines are designed to induce potent and effective tumor-specific T cell responses. Treg cells protect against autoimmunity but also suppress antitumor effector T cell responses even in the earliest neoplastic lesions. Hence, the effective state of T effector (Teff) cells and the ratio of Treg and Teff cells can define the therapeutic outcome of cancer vaccines. Recent findings suggest that inhibiting or depleting Treg cells during cancer vaccine treatment positively enhances antitumor $CD8^+$ T cell response while suppressing autoimmunity.

Treg cell depletion in metastatic breast cancer patients by an anti-CD25 monoclonal antibody, daclizumab, coupled with a multipeptide cancer vaccine resulted in robust peptide-specific $CD8^+$ T cell effector response as evidenced by specific mobilization of $CD107a^+CD8^+$ T cells and secretion of IFN-γ [8]. Here, daclizumab was administered before vaccination and acted on a subset of Treg cells via cytokine deprivation. It is plausible that CD25 blockade reprogrammed the $CD45RA^-$ but not the $CD45RA^+$ Treg cells, and these latter cells may guard against systemic autoimmunity. Furthermore, patients vigorously responded to CRM197 antigen upon vaccination with pneumococcal conjugate vaccine (PCV), despite a lack of baseline response [8]. This outcome likely represents immunological priming to CRM197 during daclizumab-mediated Treg cell depletion. In a Phase 2 clinical trial, adding a single dose of cyclophosphamide after administering the multipeptide cancer vaccine IMA901 reduced Treg cells, enhanced vaccine-induced immune responses, and extended survival of patients with renal cell cancer [9]. Since cyclophosphamide predominantly affects proliferating Treg cells, this study underscores the potential synergistic effects of depleting Treg cells after vaccination to augment anticancer vaccine responses.

Another Treg cell modulator, bempegaldesleukin (BEMPEG: NKTR-214), an engineered IL-2 cytokine prodrug, was designed to rapidly expand natural killer (NK) cells, $CD8^+$ T cells, Teff-derived IFN-γ and TNF-α, and to selectively deplete intratumoral Treg cells (Figure 1) [10]. A nonhuman, great-ape-derived adenovirus (GAd) vaccine containing neoantigens from murine colon carcinoma CT26 cells, when added to BEMPEG or a combination of anticytotoxic T-lymphocyte-associated protein (CTLA)-4 and an anti-PD-1 monoclonal antibody, led to a complete eradication of large tumors in almost all treated mice [10]. This was due to the activation and proliferation of neoantigen vaccine-specific T cells and decreased tumor-infiltrating Treg cells [10]. This triple combination therapy was also effective against MC38 murine colon adenocarcinoma cells, strengthening their potential for broader applicability. This approach opens a new avenue where other markers predominantly expressed by Treg cells, such as glucocorticoid-induced TNFR-related protein (GITR), OX-40, CD36, and CD70 can be combined with BEMPEG and checkpoint inhibitors to enhance cancer vaccine responses.

A vaccine targeting gp100 and Trp-1 melanoma antigen and OVA antigen preferentially induced cytotoxic Teff or suppressive Treg cells depending on the type of adjuvants used. Melanoma- or OVA-specific antigens adjuvanted with CpG-ODN or Poly(I:C) generated significant tumor-specific polyclonal $CD8^+$ and effector $CD4^+$ T cells [11]. In contrast, Quil A and imiquimod adjuvanted melanoma specific antigen or OVA antigen induced high suppressive Treg cells than cytotoxic $CD8^+$ and $CD4^+$ Teff cells [11]. This finding suggests a rational selection of adjuvants to overcome Treg-cell-induced immunosuppressive TME and increase vaccine efficacy.

Monoclonal antibody-mediated targeting of CCR-8 – a chemokine CC receptor critical for Treg-cell-mediated

immunosuppression – synergistically improved antitumor immune response of *Listeria*-based, live attenuated vaccine in colorectal tumor mouse models [12]. Anti-CCR8 monoclonal antibody could be combined with other modalities, including BCG vaccines and/or checkpoint inhibitors, to treat breast, colon, and lung cancer types in clinical trials. Another study showed that inhibiting the Yes-associated protein (YAP)/activin/SMAD axis in Treg cells via the drug verteporfin slowed the growth of murine B16 melanoma and EL4 thymoma. Combining verteporfin with GM-Vac (lethally irradiated B16 cells producing granulocyte–macrophage colony-stimulating factor) vaccine improved antitumor efficacy by enhancing IFN-γ producing T cells and reducing Treg cell infiltration into the TME [13]. Thus, combining Treg cell modulators after or during cancer vaccination could significantly overcome the immunosuppressive functions of Treg cells and could pave the way for an effective therapeutic cancer vaccine, resulting in tangible clinical benefits.

Concluding remarks

The role of tumor-associated macrophages and Treg cells in promoting a protumor TME has led to emerging strategies for eliminating or modulating these cells in the TME for better cancer vaccine induced antitumor response. However, not all cancers have a clear association between infiltration of Treg cells and macrophages and prognosis. Such nuances require further investigation to permit careful selection of combination therapies for each type of cancer, to avoid undesirable pathological immune responses. Given the promising, multifaceted developments presented, additional work is warranted to effectively implement targeted phenotypic modulation, in combination with cancer vaccines, as treatment for solid tumors.

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Declaration of interests

No interests are declared.

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Clinician's corner

Areas of interest to the practicing clinician include combination treatments such as the implementation of Toll-like receptor (TLR) agonists and chemokine agonists alongside cancer vaccines. These pathways ultimately increase proinflammatory cytokine production. In a murine melanoma model, which also incorporated conventional radiotherapy [2], CpG, which acts primarily at TLR9, was used in the nanoparticle construct, likely contributing to the enhanced intratumoral M1:M2 ratio, which in turn correlated with $CD8^+$ T cell infiltration. Thus far, therapies which bind TLR7, such as imiquimod, have been licensed for human use to improve antitumorigenic response in topical cancers. Broader use of such drugs is however limited by their systemic side effects. Human implementation has recently been tested using a novel TLR7 agonist DSP-0509 [14], alongside anti-PD1 immune checkpoint blockers in an unsuccessful Phase 2 clinical trial (NCT03416335), indicating the need for refinement before administration within combination therapies.

Recent murine studies highlight the potential gains of such combination therapies. Using a bispecific antibody (CD3xTRP1) alongside an imiquimod and IL-2 adjuvanted synthetic long peptide vaccine in a B16F10 melanoma murine model yielded delayed tumor growth and improved survival relative to untreated controls [15]. Importantly, TME analysis indicated that the proportion of M1 macrophages had significantly increased post-vaccination, likely due to CCR5 chemo-attractants from activated $CD8^+$ T cells. Additionally, observed survival benefits were reduced considerably when macrophages were selectively depleted using anti-CD115 antibody, re-iterating the influence of macrophages within the TME.

Once refined for human administration, such combination therapies promise to enhance polarization states within TMEs, potentially permitting more effective antitumorigenic responses to therapeutic cancer vaccines.