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Materials Based Tumor Immunotherapy Vaccines

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Abstract

Immunotherapy is a promising approach for treating cancer. However, there are limitations inherent to current approaches which may be addressed by integrating them with biomaterial-based strategies. Material platforms have been fabricated to interact with immune cells through spatially- and temporally-controlled delivery of immune modulators and to promote immune cell crosstalk. Particle vaccines have been developed to specifically target and deliver agents to organs, cells and subcellular compartments. These strategies have been shown to generate antigen-specific CTL responses and, in some cases, tumor regression. Therefore, collaboration between immunology and materials engineering is likely to result in the creation of strong vaccines to combat cancer in the future.

Introduction

Immunotherapy is rapidly becoming a standard approach for cancer treatment and management [1]. However, limitations in current approaches call for the development of more potent vaccine strategies. Over the past 30 years, the field of materials engineering has gone beyond its traditional application in medical device engineering, and biomaterials are now the basis of many drug and cell delivery, drug targeting, and tissue regeneration therapies [2]. These advances allow one to design multifunctional, versatile vehicles for molecular and cellular delivery, and these materials will likely be integrated with traditional immunotherapy paradigms in the design of next-generation vaccines against cancer. This review will first discuss current cancer immunotherapy vaccines and their limitations that may be addressed through the use of biomaterials. It will then review current efforts in material engineering to develop novel and potent cancer immunotherapies, with a particular focus on advances over the past four years.

Current cancer immunotherapy paradigm

Current cancer immunotherapy strategies target cancer cells directly or indirectly via generation of host immune cell responses to tumor antigens [1,3]. Systemic injection of monoclonal antibodies can block tumor cell function directly [4–6]. One prominent example of a monoclonal antibody therapy is the anti-HER2/neu therapy (trastuzumab) against

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invasive, HER2/neu positive breast cancer [4]. Vaccines, in contrast, typically aim to generate tumor antigen specific cytotoxic T cell (CTL) responses. Single, specific tumor antigens are often used in vaccines, and administration of tumor proteins or peptides with an adjuvant can lead to potent B cell and T cell immune responses against the particular antigen [1,7,8]. Other vaccines utilize antigens derived from whole tumor lysates, and co-administer them with adjuvants. In particular, granulocyte-macrophage colony-stimulating factor (GM-CSF) secreting, irradiated tumor cells (GVAX) have produced promising results in clinical evaluation, likely because this vaccination strategy increases the immunogenicity of a broad range of antigens [9,10].

In addition to directly priming an immune response *in vivo*, immune cells can be harvested, manipulated *ex vivo*, and re-introduced back into the body. This strategy actively stimulates a host antitumor response and provides opportunity for autologous cell activation [11]. Such vaccines include adoptive T lymphocyte transfer, in which antigen-specific reactive T cells are harvested from the patient, activated and expanded *in vitro*, and re-administered into the patient to exert tumor killing effector functions, and dendritic cell (DC) based vaccines [12,13]. In 2010, the first DC based therapeutic vaccine, Provenge, was approved by the FDA [11]. In this vaccine, a patient's own peripheral blood mononuclear cells (PBMCs) are first harvested and isolated; they are then exposed to a fusion protein composed of GM-CSF to activate antigen presenting cells (APCs) and prostatic acid phosphatase as a tumor-associated antigen. The antigen-pulsed APCs are then administered back into the patient [11]. In the body, these APCs can generate tumor specific CTLs following homing to the lymph nodes (LNs) and prime naïve T cells.

All of these cancer immunotherapy strategies have shown promise in the clinic, and some have received FDA approval, but there are a number of limitations inherent to these approaches. First, most systemically administered vaccines lack cell specificity and have short signal duration. Multiple administrations are often needed, leading to associated dose limiting systemic toxicity [14]. Second, cell-based vaccines are costly and have shown limited effectiveness, as indicated by modest increases in patient survival to date [11]. It is possible that a large percentage of reinfused cells die after transplantation, and even fewer retain their effector functions [15]. Lastly, these vaccines generally lack the ability to target subcellular compartments to control adjuvant delivery and antigen processing, which limits their ability to induce optimal T cell priming. For example, endocytosed soluble antigens are inefficiently cross-presented by DCs, a process shown to be important for priming a strong CTL response [16].

Incorporating biomaterial technologies into immunotherapies may overcome limitations of current cancer immunotherapies, as biomaterials can be designed to target and control cells from disparate organ systems. Materials-based technologies have a number of attractive features. First, these technologies are often multifunctional; multiple immune modulators, including antigens and adjuvants, can be loaded onto a single material system [17]. Second, they can target specific cell populations and deliver payloads to these cells, potentially reducing high dosage related systemic toxicity (Fig 1a). Third, they can promote cell-cell interactions and crosstalk (Fig. 1b). Fourth, they can promote durable systemic responses by protecting pre-loaded agents from degradation and present signals over long time frames to host immune cells. Lastly, they can exploit our knowledge of cell biology and chemistry to manipulate cell function. In this review, several recent materials-based vaccine strategies, including the use of polymer scaffolds to direct immune cell function, micro- and nanoparticles to specifically target relevant organs, cells and subcellular compartments, and nanoparticles to engineer immune cells, will be discussed.

Biomaterials to modulate immune cell trafficking and activity

One main goal of cancer immunotherapy is to tip the balance from tumor tolerance to tumor immunity [3]. Biomaterials allow one to create a microenvironment to actively educate immune cells to break tolerance *in situ* to exert anti-tumor immunity. Three dimensional porous scaffolds have received much attention in regenerative medicine and tissue engineering research for their ability to deliver growth factors and cells with spatiotemporal specificity, to promote cell-cell interactions in the matrix, and to direct cell function [2,18,19]. This functionality is similarly useful in the development of tumor vaccines that program host immune cells and allows these biomaterials to replace current immunotherapies that manipulate immune cells *in vitro*. For example, biodegradable scaffolds fabricated from poly(lactide-co-glycolide) (PLG) have been developed over the past few years for this purpose (Fig. 2a), as PLG is a widely used and biocompatible medical polymer [19–21]. The PLG scaffolds were loaded with an immature DC chemoattractant, GM-CSF, and decorated with condensed CpG, a TLR9 agonist. Melanoma tumor lysate was utilized as the cancer antigen. The gradient of local GM-CSF that resulted from its sustained and localized release led to the recruitment of a high number of immature DCs to the scaffold (Fig. 2b). There, the DCs were activated by CpG (Fig. 2c) and processed the tumor antigen. Activated DCs then homed to the draining lymph nodes (dLNs) with high efficiency and primed naïve T cells. This vaccine induced 90% prophylactic tumor protection and more strikingly, also generated therapeutic protection, as complete regression of established melanoma was observed in a fraction of the animals (Fig. 2d). The concept of generating a local immunogenic niche to promote immune cell crosstalk was demonstrated also in an alginate-based vaccine [18]. Here, peptide-loaded and activated DCs were transplanted using an alginate matrix. This alginate “node” attracted antigen specific T cells and host DCs for over a week, and led to antigen-specific CTL expansion. In both cases, the material scaffold remained at the site of vaccination and generated durable immunogenic responses; the PLG scaffold remained active for months post implantation, which cannot be achieved by any means of soluble administration to date.

Micro and nanoparticles to target and engineer organs and cells

An alternative approach to recruiting and programming immune cells within a biomaterial is to instead use engineered materials that directly target immune cells. This increased specificity may reduce high dosage related systemic toxicity common to current vaccines. In particular, micro- and nanoparticles have been utilized to target immune cells in the lymph nodes (LNs), mucosal tissues, and the tumor microenvironments. Particle targeting depends highly on particle size. Post intravenous injection, particles between 500 and 2000 nm are generally processed by APCs at the injection site, whereas smaller nanoparticles, 20–200 nm, can traffic to the LNs where they are processed by LN-resident DCs [22–24]. These particles can be synthesized using a wide range of materials, and antigens and adjuvants can be conjugated to the particles prior to delivery by exploiting the chemistry and physics of the materials [25–31]. For example, a multifunctional nanoparticle system that simultaneously delivers carcinoembryonic antigens (CEA) and allows imaging of the LN using MRI was recently developed (Fig. 3) [29]. Here, iron oxide-zinc oxide ($\text{Fe}_3\text{O}_4\text{-ZnO}$) nanoparticles (16 nm) (Fig. 3a) carrying the CEA were injected in the footpad, trafficked to the dLNs (Fig. 3b), and were internalized by the DCs. This process could be readily imaged *in vivo* using MRI (Fig. 3c) due to the properties of the iron oxide-zinc carrier. Mice that were vaccinated with this nanoparticles system showed a strong CTL response and significant tumor growth reduction (Fig. 3d–e). Microparticles are typically designed not for rapid up-take by APCs, but instead for local delivery of biological agents [30,32]. For example, microparticles have been pre-loaded with poly(I:C) and directly injected into the lymph node along with soluble antigens [30]. There, they prolonged DC activation and enhanced antigen processing. Other

microparticles have been designed to mimic mast cell degranulation with slow release of their contents. In this case, chitosan-heparin nanoparticles encapsulating cytokines such as TNF or IL-12 were injected intravenously, and released the cytokines in a sustained and controlled manner into the LNs. This vaccine induced a prolonged and potent systemic T_H1 response. In both cases, it is likely that microparticle-based vaccines prolong immune cell action by protecting the cytokines from degradation until release.

In addition to LN targeting, nanoparticles can be used to target mucosal tissues, where many primary tumors originate, and specific immune cells in the tumor microenvironment. Being able to specifically target mucosal immune cells is attractive because systemic vaccination is ineffective at generating adequate immune responses at these sites. Mucosal tissue targeting has focused on respiratory and reproductive tissues [33–36]. For example, when nanoparticles are directly administered to the lungs, they were actively taken up by pulmonary DCs [34]. These DCs then trafficked to the draining LNs, and generated high pulmonary and systemic CTL responses capable of protecting mice from an ovalbumin-influenza infection. As nanoparticles concentrate within and penetrate tumors, they can target selected subsets of cells in the tumor environment. There, they can promote immune cell crosstalk locally to exert tumor killing effects [37–39]. For instance, liposomal nanoparticles conjugated with anti-CD40 and CpG have been used to target and activate the APCs in tumors [37]. When compared to the soluble form of the vaccine, the nanoparticle-based vaccine reduced systemic presence of anti-CD40 and CpG, reduced systemic levels of inflammatory cytokines, and slowed growth of tumors.

Nanoparticles to manipulate subcellular compartments

Nanoparticles can also be designed to manipulate subcellular compartments in order to improve APC antigen presentation, and potentially induce antigen cross presentation. As nanoparticles are endocytosed into endosomes and phagosomes, many strategies exploit the acidic and reductive environment in these cellular compartments. The use of pH- and oxidation-reduction-dependent chemistry to load adjuvants and antigens can result in payload release only upon nanoparticle endocytosis [40–43]. For example, an interbilayer-crosslinked multilamellar vesicle (ICMV) vaccine (Fig. 4) was developed in which the liposome layers were embedded with monophosphoryl lipid A (MPLA), and antigens were encapsulated inside the liposomes (Fig. 4a) [44,45]. This design demonstrated a number of advantages of nanoparticle systems. First, the nanoparticles size (about 250nm) was sufficiently small to traffic to and accumulate in the LNs. Second, the multi-modular design ensured that the same APC will uptake MPLA and the antigen together upon internalizing a nanoparticle. Third, the thiol bond between the lipid layers is stable in physiological conditions but will destabilize in response to the enzymes that are present in the endolysosomes. This results in rapid degradation of the carrier in the endosome and release of the entrapped antigen (Fig. 4b). Though a specific cross presentation mechanism was not elucidated, this vaccine strategy was able to induce impressive antigen specific CTL and humoral responses (Fig. 4c–d). Nanoparticles have also been designed to directly perturb the endosomal membrane to “pump” antigens into the cell cytoplasm, although this effort is still in its nascent stage. Nanoparticles for this purpose can be synthesized to include a photosensitizer [46,47]. Once a cell has endocytosed the loaded nanoparticle, an external optical trigger can be applied, disrupting the endolysosome membrane and causing a burst release of its entire contents. Other technologies can deliver nanoparticles directly into the cytoplasm, bypassing the endosomal pathway altogether [48]; this potentially increases the chance of intact antigens being processed by the Class I MHC pathway.

Nanoparticles to engineer cells *ex vivo*

In cell based therapies, such as adoptive transfer of T lymphocytes, *ex vivo*-manipulated cells are administered with modulators to enhance cell survival and function. These modulators may need to be maintained at high levels for an extended time for the therapy to be efficacious, and because of their pleiotropic effects, they may generate systemic toxicity [49,50]. One approach to overcome these issues is to directly conjugate modulator-containing nanoparticles to the cell surface before re-infusion [50]. For example, liposome nanoparticles containing IL-15 α and IL-21 have been tethered to the surface of autologous melanoma-specific T lymphocytes. The fundamental functions of these lymphocytes were unaffected, but nanoparticle-tethering greatly enhanced their survival and systemic circulation. Mice vaccinated with this approach showed slower tumor growth and complete tumor protection. Using the same principle, nanoparticles containing NSC-87877, an inhibitor of Shp1 and Shp2, were tethered to T lymphocytes [49]. These engineered T lymphocytes underwent significant expansion at the tumor site following adoptive transfer, enhancing survival in a model of prostate cancer. In both cases, materials-based *ex vivo* manipulation of autologous cells greatly increased their survival and effector functions.

Conclusions and future directions

Materials engineering provides a number of attractive new strategies to design more potent tumor immunotherapy vaccines. Material platforms have been created to interact with DCs through spatial-temporal control over delivery of growth factors, adjuvants, and antigens, and to promote immune cell crosstalk. Micro- and nanoparticles have been shown to specifically target organs, cells and subcellular compartments to deliver cytokines, siRNA, adjuvants, antigens, and other modulators.

The field of materials-based cancer vaccination is still in an early stage, much still remains to be learned, and significant advances remain to be made. The biocompatibility and safety of many of the materials used in new vaccines must be addressed. Because materials-based vaccines are often multifunctional and multi-modular, thorough and systematic investigations are necessary to examine their mechanism(s) of action and impact on the immune system. Materials-based vaccines are capable of delivering a large set of tumor antigens, instead of only single defined antigens, and will allow the importance of this approach to be systemically explored. The advantage of using multiple antigens, or whole tumor as the antigen source, is that the resulting immune response is mounted against multiple possible tumor antigens. Future studies will also likely explore multi-adjuvant delivery, crosstalk between different immune cells, specific targeting of subsets of immune cells, more effective strategies to promote and demonstrate antigen cross presentation, and improved spatial and temporal control over delivery of anti-tolerance factors. Recent clinical studies have produced exciting results in tumor immunotherapy, and material technologies may lead to further improvements in patient response in the future. A tight collaboration between immunology and materials engineering is likely to result in the creation of multiple future generations of vaccines to combat cancer.

Acknowledgments

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* of special interest

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Highlights

- Current tumor immunotherapy may be improved by integrating with material-based strategies.
- Material-based vaccines induce strong immune responses and tumor regression.
- Material platforms can interact with and direct immune cells to exert anti-tumor functions.
- Particle vaccines can target and manipulate organs, cells and subcellular compartments.

Figure 1. Schematics of materials-based tumor immunotherapy strategies

(a) Particles vaccines can specifically target (1) various organs, such as the LN, mucosal tissues and the tumor, as well as (2) particular cell types and subcellular compartments. (b) Material platforms can be used to recruit immune cells to the material, and direct cell function (such as anti-tumor effector functions) through cell-material and cell-cell interactions in the matrix, and by locally concentrating immune modulators and presenting to recruited cells.

Figure 2. PLG based 3D polymer scaffold vaccine to recruit and program immune cells to generate anti-tumor immunity (ref 19, 20)

(a) Overall schematic of the vaccine. 1. SEM image of a PLG 3D scaffold; scale bar: 1000 μm (b) Number of CD11c⁺ DCs isolated from the scaffold on day 14 post implantation in response to 0, 400 ng, 3000 ng and 7000 ng of GM-CSF. (c) The number of CD11c⁺CCR7⁺ DCs isolated from the scaffold loaded with PEI-ODN control, 10 μg PEI-CpG-ODN, 400 and 3000 ng GM-CSF and 400 and 3000 ng GM-CSF in combination with 10 μg PEI-CpG-ODN at day 7 after implantation. (d) A comparison of the survival of mice following prophylactic (left) and therapeutic (right) vaccination. In the prophylactic vaccination, mice were vaccinated with blank PLG scaffolds (Blank), antigen+ 100 μg CpG-ODN (Lys +100CpG), antigen+3000 ng GM-CSF+10 μg CpG-ODN (Lys+3000GM+10CpG), antigen +3000 ng GM-CSF+100 μg CpG-ODN (Lys+3000GM+100CpG), or irradiated, GM-CSF-transduced B16–F10 cells (cell based). Mice were challenged (day 0 on graphs) with 10^5 B16–F10 melanoma tumor cells and monitored for the onset of tumor occurrence. In the therapeutic vaccination, mice were (inoculated with 5×10^5 B16–F10 cells and allowed to develop for 9 days and treated with blank PLG matrices (Blank), PLG matrices loaded with 3000 ng of GM-CSF and 100 mg of CpG-ODN (GM+CpG). Mice were also treated once (Vax, 1 $\times$; at day 9), or twice (Vax, 2 $\times$; at days 9 and 19) with PLG matrices incorporating GM-CSF, CpG-ODN, and tumor lysates (Vax). Mice were also treated with 5×10^5 irradiated, GM-CSF–transduced B16–F10 cells. Panels (c) and (d left) reproduced with permission (ref 19), Copyright 2009, Nature Publishing Group. Panels (b) and (d right) reproduced with permission (ref 20), Copyright 2009, The American Association for the Advancement of Science.

Figure 3. Fe_3O_4 - ZnO nanoparticles for specific targeting and imaging of LNs (ref 29)
 (a) Schematic of nanoparticle with conjugated CEA. (b) Representative immunohistochemistry of draining lymph node 48 hours after footpad injection with Fe_3O_4 - ZnO nanoparticle-labeled DCs (dark brown dots). The upper panel is stained with anti-Thy1.2; the lower panel is stained with anti-B220. (c) *In vivo* MRI images of dLNs of a mouse (left) injected with DCs labeled with Fe_3O_4 - ZnO (red arrow) or ZnO nanoparticles (yellow arrow) 48 hours after footpad injection. Right shows a dLN (green arrow) of cell-free Fe_3O_4 - ZnO nanoparticle-injected mouse. (d) CEA-specific cytotoxic activity of splenocytes from immunized mice. Mice were immunized with DCs loaded with nanoparticle/3 ZBP-CEA (open red squares), nanoparticle/CEA (filled red squares), 3 ZBP-CEA (open blue triangles), CEA (filled blue triangles), nanoparticle (open black circles) or DC only (filled black circles). MC38 cells with (MC38/CEA) or without (MC38) CEA expression were used as targets. (e) Tumor volume (left) and survival rate (right) of mice injected with MC38/CEA cells. Mice were immunized as in (d), four times at weekly intervals starting one week after tumor injection. Reproduced with permission, Copyright 2011, Nature Publishing Group.

Figure 4. ICMV vaccine for LN targeting and controlled antigen and adjuvant delivery (ref 45)
 (a) Schematic illustration of ICMVs. Soluble OVA mixed with MPLA (MPLA), OVA-loaded ICMVs with MPLA only on the external surface (ext-MPLA ICMVs) or OVA-loaded ICMVs with MPLA throughout the lipid multilayers (int-MPLA ICMVs) (b) Release of OVA from ICMVs was measured after 1 day at 37°C in buffers simulating different aspects of the endolysosomal environment. (c) Frequency of OVA-specific T cells in peripheral blood, assessed over time, as indicated by tetramer⁺CD8⁺ T cells. Mice were vaccinated with ICMVs containing 10 g OVA, and 0:1 g MPLA externally (ext-) or throughout the multilayers (int-). (d) OVA specific serum IgG level on day 56 in mice vaccinated on day 0 and boosted on days 21 and 35 Mice were vaccinated with 0.1 µg or 1 µg MPLA, in soluble form or in ICMVs, externally (ext-) or throughout the multilayers (int-). Reproduced with permission, Copyright 2011, Nature Publishing Group.