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Tumor lysate-loaded biodegradable microparticles as cancer vaccines

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Abstract

Cancer vaccines that use tumor lysate (TL) as a source of tumor-associated antigens (TAAs) have significant potential for generating therapeutic anti-tumor immune responses. Vaccines encompassing TL bypass the limitations of single antigen vaccines by simultaneously stimulating immunity against multiple TAAs, thereby broadening the repertoire of TAA-specific T cell clones available for activation. Administration of TL in particulate form, such as when encapsulated in biodegradable microparticles, increases its immunostimulatory capacity and produces more robust immune responses than when TL is given in soluble form. These effects can be further enhanced by co-administering TL with adjuvants. A number of recent studies using polymeric microparticle delivery of TL, with or without adjuvants, have produced promising results in preclinical studies. In this review, we will discuss current experimental approaches involving TL being pursued in the oncoimmunology field, and comment on strategies such as combining specific chemotherapeutic agents with TL microparticle delivery that may eventually lead to improved survival outcomes for cancer patients.

Cancer vaccines in principle and practice

The identification of TAAs was a major breakthrough in the development of cancer vaccines and immunotherapies [1]. Numerous strategies for using TAAs in immunotherapies have been tested, with the common goal of stimulating anti-tumor immune responses that lead both to tumor clearance and to the generation of memory cells that can protect against future tumor recurrence. There is now abundant evidence to demonstrate that significant regression of tumors can be achieved by stimulating TAA-specific CD8⁺ cytotoxic T lymphocytes (CTLs), and that, importantly, cancer patients harbor TAA-specific T cell clones capable of mediating tumor cell killing if given the appropriate stimuli [2–4]. As a result, most cancer vaccines are currently designed towards the *in vivo* activation of TAA-specific CTLs by dendritic cells (DCs) that have been treated with a combination of TAAs and immunoadjuvants. Mature DCs can provide multiple signals to naive CD8⁺ T cells that induce their expansion and differentiation into CTLs. These signals include cognate peptide-MHC complexes on DCs interacting with the T cell receptor, co-stimulatory receptor/ligand interactions, and an appropriate cytokine milieu that can influence the nature of the immune

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response (e.g. a Th1-biased cytokine profile favoring CTL activation). The generation of effective immune responses is often impeded by tumor cells, which typically promote immunosuppressive cytokine production, regulatory T cell (Treg) activity, and accumulation of a variety of myeloid-lineage suppressor cells (e.g. myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory tumor-infiltrating dendritic cells) both within tumors and systemically [5–8].

To reduce the impact of these inhibitory networks, much research has focused on more efficient loading and activation of stimulatory DCs, both *ex vivo* and *in vivo*, in order to increase the potency of downstream T cell anti-tumor immunity. For prostate cancer, the approach used by the recently FDA-approved sipuleucel-T® (Dendreon Corporation, Seattle, USA) therapy involves *ex vivo* maturation and antigen-loading of autologous DCs with prostatic acid phosphatase fused to granulocyte-macrophage colony stimulating factor (GM-CSF) prior to adoptive transfer back into cancer patients [9]. As new variations of this strategy are developed in animal models, a major challenge for clinical application is the selection of an appropriate TAA for each type of cancer and, in certain situations, each cancer patient. Tumors comprise a heterogeneous population of cells that express multiple TAAs at varying levels; furthermore, tumors frequently undergo mutagenic shifts that alter the composition and antigenicity of TAAs [10]. Therefore single-antigen vaccines can fail to effectively prevent tumor growth despite achieving detectable, systemic TAA-specific CTL responses, whilst improved clinical responses have been seen when multiple TAAs are used in cancer vaccines [11].

For these reasons, many new vaccine strategies being investigated include co-delivery of multiple TAAs with or without adjuvant(s) to DCs *in vivo* [12]. Vaccines containing preparations of whole tumor cells or TL have the advantage of simultaneously presenting multiple defined and undefined TAAs to the immune system. These strategies can obviate the need for expensive and laborious *ex vivo* manipulations of the patient's own DCs and tumor cells. Apoptotic whole tumor cells inherently present a large amount of tumor material in discrete packages and may have an advantage over TL *per se* when comparing their abilities to be phagocytosed by DCs and induce DC maturation. However, by using well-defined and relatively simple manufacturing processes, TL can be encapsulated into discrete, biodegradable polymer-based microparticles that possess predetermined and customizable characteristics such as size, release kinetics, adjuvant content, and targeting moieties. From this point onward, we will focus on current progress in the development of microparticle-based cancer vaccines that promote anti-tumor immunity by delivering TL to DCs.

Multi-antigen tumor lysates in vaccines

Both TL and whole-cell tumor vaccines possess multiple TAAs, thereby expanding the repertoire of both CD4+ (helper) and CD8+ (cytotoxic) T lymphocytes that are activated. This consequently decreases the possibility of immune evasion by the tumor. However, vaccines using single TAA have shown promising results in clinical trials. The downside of single-antigen vaccination is a limited potency of the desired anti-tumor CTL responses, as only a narrow range of CTL clones can be recruited to perform the task of tumor cell clearance. Ultimately, immune evasion through the development of tumor antigen loss variants is more readily experienced. The phenomenon of immune-mediated antigen loss in tumors has been reported in multiple human and murine studies [13–15]. For example, a subset (20 – 30%) of patients with glioblastoma multiforme (GBM), an aggressive and incurable form of brain cancer, have shown to be promising candidates for vaccination with a tumor-specific peptide, PEPvIII(-KLH). This peptide is derived from a mutated form of the epidermal growth factor receptor, called variant III (EGFRvIII) [13]. Phase II clinical

trials revealed that patients with EGFRvIII-expressing GBM who received multiple intradermal vaccinations of PEPvIII-KLH plus GMCSF and chemotherapy (temozolomide) had enhanced overall survival over historical controls. However, over 90% of recurrent tumors in treated patients were EGFRvIII-negative. In a separate phase I study of patients with recurrent GBM, 4 of 9 subjects who were vaccinated with autologous TL-pulsed DCs had increased levels of one or more CD8+ T cell clones specific for the TAAs MAGE-1, gp100, and HER-2 [16]. In addition, the median survival for vaccinated patients versus a matched control group was 133 weeks versus 30 weeks ($p = 0.0013$). A subsequent phase II trial involving a similar treatment regime, established a logarithmic correlation between vaccine-induced immune responses (IFN- γ production) and clinical outcomes (e.g. post treatment times to survival) [17].

Another advantage of using multi-antigen TL vaccines is that, in theory, their clinical use would not be restricted to cancer patients who express a specific TAA or MHC haplotype [18]. TL-based vaccines also bypass the expensive and laborious task of purifying single antigens for use in vaccines. In an attempt to investigate the importance of single antigen versus multiantigen based vaccines Neller *et al.* performed a meta-analysis of various published cancer immunotherapy clinical trials, and reported that on average, 8.1% of patients vaccinated with whole tumor or tumor extracts displayed objective clinical responses, whereas only 3.6% of patients responded to molecularly-defined single antigen-based therapies [11]. The disadvantage of using whole tumor cells or TL for vaccination is that the ensuing immune response may be more difficult to evaluate, as CTL responses develop to numerous undefined TAAs. With single-antigen vaccinations, investigators can track the increased frequency of one population of TAA-specific CTLs with tetramer analysis, and this approach does provide an easy read-out for one aspect of the anti-tumor immune response. However, there are mixed reports on whether the frequency of circulating tumor antigen-specific CD8 T cells correlates with overall survival, so using systemic T cell frequency as a surrogate read-out for survival should be viewed with caution [19–23]. Thus, for both TL or single-antigen vaccinations, it appears that overall survival is still the best validation of therapeutic success.

The use of TL in cancer vaccines was first trialed in melanoma patients, generating initially promising results with 5/17 patients displaying complete or partial responses in a phase I clinical trial [24]. Known as Melacine® (Corixa Corporation) this allogeneic melanoma TL vaccine was administered with an adjuvant, DETOX, a water-in-oil emulsion similar to incomplete Freund's adjuvant (IFA). Follow-up phase II and phase III clinical trials resulted in only modest overall objective clinical responses (6 – 7%) (reviewed by [25]). One possible explanation for the modest anti-tumor effect is that the adjuvant used, being similar to IFA, may have promoted Th2-biased immune responses rather than Th1-biased immune responses, the latter of which is a more desired outcome for cancer vaccines [26]. Reassuringly, it has been demonstrated that TL-pulsed human DCs can, though not always, generate Th1-biased immune responses [27,28]. In addition, promising results have been achieved in patients with malignant glioma vaccinated with autologous DCs pulsed with autologous TL, with median survival of patients being increased from 30 weeks (control group) to 133 weeks [16]. However, a rare *in vitro* study that compared the immunostimulatory ability of human DCs pulsed with apoptotic tumor cells (DC/APO) versus DCs pulsed with TL (DC/TL) found that DC/APO stimulated significantly stronger CTL responses than DC/TL when co-cultured with autologous peripheral blood mononuclear cells [29]. Since the authors concluded that neither DC/APO nor DC/TL promoted DC maturation, it is possible that the more immunostimulatory nature of DC/APO could be due to the delivery of the tumor material in the form of discrete packages rather than in soluble form, thereby promoting more efficient uptake and/or cross-presentation. Studies such as these, along with results from clinical trials, illustrate that there is still a dire

need for improvement where TL vaccines are concerned. The use of soluble TL in principle suffers from a number of flaws which include rapid antigen disintegration, inefficient uptake and cross-presentation of antigen by DCs, and the often reported inability of TL to promote DC maturation thus leading to T cell tolerance [30,31]. One strategy for overcoming these flaws is to encapsulate TL in biodegradable polymeric microparticles, as this may not only improve the uptake efficiency of TL by host DCs but may potentially also promote cross-presentation of TAAs thereby enhancing the activation of naive TAA-specific CD8+ T cells [32,33].

Particulate delivery of TL to DCs *in vitro*

Biodegradable polymer particles prepared from natural or synthetic polymers such as chitosan and poly(lactic-co-glycolic acid) (PLGA) can be used to deliver surface adsorbed or encapsulated TAAs to DCs [34–37]. The delivery of microparticulated TL (or TAAs in general) to DCs provides many advantages over administering TL in soluble form. For example, the particulate nature of biodegradable microparticles can mimic the shape and size of microbes, thereby facilitating uptake and processing by DCs [38]. In addition, encapsulating TL in particles preserves the stability of antigens, often establishes a depot, and consequently generates more robust immune responses. Prasad *et al.* found that human DCs, loaded with PLGA-encapsulating TL, were capable of triggering significantly greater interferon (IFN)- γ production and significantly lower interleukin (IL)-10 production in autologous CD8+ T lymphocytes than when DCs were loaded with soluble TL [31]. Importantly, the DCs, the TL, and the CD8+ T lymphocytes were derived from cancer patients (with head and neck squamous cell carcinoma), revealing their potential for stimulating endogenous anti-tumor immune responses. Similarly, Hanlon *et al.* demonstrated that DCs derived from healthy human donors and pulsed with PLGA particles encapsulating TL from an epithelial ovarian cancer cell line stimulated production of significantly higher levels of Th1 cytokines (e.g. IL-1 β , IL-6, IFN- γ) by co-cultured autologous CD8+ T cells when compared to DCs pulsed with soluble TL [39]. In fact, DCs pulsed with soluble TL often promoted a Th2-like cytokine profile, upregulating IL-4 and GM-CSF expression. In addition, effector T cells were generated when particulated TL was used whilst exhausted/tolerized T cells were generated when soluble TL was used. These data indicate that microencapsulation can avert the problem of TL-induced tolerance.

Many other biodegradable microparticle systems, aside from PLGA, have the potential to be used as vaccine vectors for TL but have, for the most part, not yet been tested either clinically or preclinically. These include various forms of liposomes, chitosan, amphiphilic particles, acid degradable hydrogels, and gelatin particles, all of which have been reviewed in terms of their cancer vaccine potential elsewhere [34]. In one study that used liposomes loaded with tumor lysates as potential cancer vaccines, it was shown that fusogenic liposomes, loaded with TL (from murine B16BL6 tumor) were more efficient at providing prophylactic protection against B16 tumor challenge when compared to conventional liposomes loaded with the same TL [40]. It was proposed that the increased immune efficacy of the fusogenic liposomes was due to a combination of the ability of the fusogenic liposomes, which are a hybrid of conventional liposomes and inactivated Sendai virus, to stimulate DC maturation and promote cross-presentation of TAAs. Unfortunately the researchers found that the fusogenic liposomes (loaded with TL) had no therapeutic effect, suggesting that the potency of the immune response was inadequate.

Co-delivery of TL plus adjuvant enhances anti-tumor immune responses *in vivo*

Mature DCs that efficiently process and present TAAs are critical for inducing systemic CTL responses with potent anti-tumor activity [41–43]. DCs act as sentinels in tissues, maintaining peripheral tolerance to self-antigens while initiating adaptive immune responses against foreign antigens. Most TAAs are recognized by the immune system as self, rather than foreign. Therefore triggering immune responses to TAAs requires the breaking of peripheral tolerance, usually by the introduction of an adjuvant to the vaccine formulation that provides immunologic “danger signals” [44]. Danger signals can be viral or bacterial components or synthetic derivatives that often bind Toll-like receptors (TLRs), triggering DC maturation [45–47]. However, administration of TAA and adjuvant mixtures in solution does not guarantee the co-delivery of these molecules to the same DC, potentially leading to sub-optimal immune responses [48,49]. To overcome this limitation, microparticle vaccine systems have been designed to co-administer TAAs and adjuvant in a single delivery vehicle thus preventing independent uptake of TAA and adjuvant by DCs [50–52]. Consideration should also be given to the families of inducible heat shock proteins (e.g. calreticulin and HSP90), which can be a source of endogenous danger signals capable of promoting tumor antigenicity through a range of mechanisms that include enhanced cross-presentation of antigens on DC MHC Class I (reviewed by [53]). Aside from inducing HSP expression endogenously prior to lysis, these proteins could also be added to tumor lysates in defined quantities prior to encapsulation.

The potential benefits of microparticle co-delivery of TAAs plus adjuvant has been demonstrated in murine studies. In one murine vaccination study PLGA microparticles were co-loaded with the model TAA, ovalbumin (OVA), plus the adjuvant, CpG (an oligodeoxynucleotide containing unmethylated CpG motifs that ligates TLR-9) [54]. Vaccination with these microparticles resulted in enhanced *in vivo* OVA-specific CTL activity as well as enhanced tumor protection relative to OVA-loaded particles delivered in combination with a solution of CpG. In another study, PLGA particles were prepared that co-encapsulated CpG plus TL prepared from transgenic mice with prostate adenocarcinoma (the TRAMP strain [55]). Therapeutic vaccination with these particles promoted anti-tumor activity when supplemented with a TLR-3 agonist [56]. Another advantage of this approach is that encapsulation of TAA and adjuvants in biodegradable microparticles limits local release of these molecules during administration, as excessive release can induce unwanted inflammation at the injection site due to prolonged local accumulation and activation of immune cells as observed with administration of TAA emulsified in IFA [57].

Prospective combinatorial vaccine strategies

As is the case with cancer vaccines in general, combining TL-loaded microparticle vaccine therapy with strategies to eliminate immune suppressor cells can further amplify protective CTL responses *in vivo*. As evidence that such an approach is worth pursuing, we found that mice vaccinated with PLGA particles co-encapsulating melanoma TL and CpG were afforded enhanced protection from subsequent tumor challenge when Tregs were depleted using a systemically administered anti-CD25 monoclonal antibody [58]. Targeted removal of immunosuppressive cell populations can be accomplished through methods other than antibody depletion, and many of these are well-suited to adaptation in microparticle-based therapies. Several recent studies have shown that low-dose chemotherapy can also deplete immunosuppressive cell populations, thereby enhancing protective CTL responses [59,60]. More specifically, we and others have shown that 5-fluorouracil and clodronate can deplete MDSCs and TAMs, respectively [61–64]. Another benefit of using cytotoxic drugs as part of a combinatorial vaccination strategy is that some agents have been shown to improve the

immunogenicity of cancer cells undergoing apoptosis. For example, therapeutic treatment of tumor-bearing mice with doxorubicin led to an enhanced prevalence of CD8+ T cells in tumor-draining lymph nodes and suppression of tumor growth [65]. Overall, these observations suggest that the addition of agents that directly or indirectly promote the effector arm of the immune response may substantially enhance the anti-tumor benefits of microparticle-based TL vaccines. Frazier *et al.* have summarized the outcomes of combining active specific immunotherapy (including TL vaccines) with chemotherapy in recent preclinical and clinical studies, documenting its promising potential for a range of cancers [66]. In the future, more sophisticated microparticle formulations may be manufactured that simultaneously provide agents that promote immune effector responses (e.g. encapsulated TL plus adjuvant(s)) and dampen Treg activity (e.g. microparticles coated with Treg antagonists). Altering the formulations of microparticles that result in differential release patterns of TAAs versus chemotherapeutic agents may also prove therapeutically beneficial since it has been observed that staggering these treatments has enhanced anti-tumor activity [67].

Conclusions

There are numerous potential advantages associated with delivery of TL encapsulated in biodegradable microparticles over other cancer vaccine systems. These include: (i) enhanced immunogenicity of encapsulated versus soluble TL due to prolonged presence of the antigenic material as well as enhanced uptake and cross-presentation by DCs, (ii) co-delivery of antigen and adjuvant to the same DC, thereby promoting DC maturation; (iii) delivery of multiple TAAs to enhance the magnitude of the protective immune response. In addition, as is the case for cancer vaccines in general, there exists the potential to further enhance anti-tumor immunity by combining TL vaccination with simultaneous depletion of specific immune suppressor cells. Finally, the ease with which the composition of polymer particle-based vaccines can be modified to achieve clinical efficacy is an attractive feature that could ultimately benefit TL vaccines therapeutic efficacy. Although more optimization remains to be done in this field, pre-clinical results in murine tumor models have shown the tremendous potential for this therapeutic approach.

Expert commentary

Establishing effective treatment strategies for cancer is still one of the many daunting challenges in the medical arena. Most cancer patients diagnosed with advanced stage disease have an extremely poor prognosis. Valuable discoveries over the past 2 decades have given impetus to the field of oncoimmunology; these include the identification of TAA-specific T cell clones within cancer patients, as well as the discovery of Toll-like receptors and their ligands. Using biodegradable polymers as vectors for tumor lysate vaccines offers the potential for improvement over the delivery of soluble tumor lysate vaccines as well as over vaccines that involve only single antigens. Co-encapsulation of antigen and adjuvant into microparticles ensures co-delivery of these components to the same DC in a form considered to mimic infection with intracellular pathogens, which leads to desired Th1-biased immune responses. Although the full therapeutic potential of TL vaccines and cancer vaccines in general is as yet untapped, it is likely that this potential may well be realized with more sophisticated formulations and combinatorial treatment approaches.

Five-year view

Small but significant advances can be foreseen over the next 5 years for cancer vaccines involving microparticulated tumor lysates. Overcoming the theoretically legitimate concern that TL and whole tumor cell vaccines will lead to autoimmunity is one achievable

milestone, since recent clinical trials are proving that such vaccines are generally well-tolerated by patients. The accumulation of both clinical and preclinical data over the next 5 years should hopefully establish the safety of TL vaccines. What is of more concern are scenarios where TL vaccines are combined with treatments that dampen the immunosuppressive arm (e.g. Tregs) of the immune response, as it is then more likely that serious autoimmune effects will become apparent. For such combinatorial therapies, it will be necessary to carefully fine-tune immunosuppressive components, but ultimately this will have to be determined empirically in clinical rather than preclinical settings. Therefore, expecting definitive outcomes within 5 years as to optimal combinations of TL vaccines with other treatments may be too optimistic. However, the accumulation of data from clinical studies would nevertheless be of great value and would point clinicians in the right direction. In regards to the polymer-based microparticles used to encapsulate TL, the predominant polymer used thus far has been PLGA and we would expect that over the next 5 years there will be more research into using a variety of other biodegradable polymers. The capacity for microparticles to efficiently co-deliver antigen and adjuvant to the same DC *in vivo* may also supercede the need to treat DCs *ex vivo*, thereby avoiding the extensive manual labor involved in generating DC-based vaccines.

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Key Issues

- Tumor cells express a cocktail of defined, and in all likelihood, undefined tumor-associated antigens (TAAs) which could be potential targets for the host's immune response.
- Tumor lysate (TL), as the antigenic component of a cancer vaccine, can provide multiple TAAs as immune targets thereby decreasing the possibility of immune evasion by the tumor through epitope loss or through intratumoral heterogeneity.
- TL vaccines involving either autologous or allogeneic tumor lysates increase the patient eligibility rate over single TAA-based vaccines by obviating the restriction imposed by HLA phenotypes.
- Particulated delivery of TL using biodegradable polymer formulations provides many benefits that include promotion of co-delivery of TL and adjuvant to the same dendritic cell.
- Various types of biodegradable polymers could potentially be used as delivery systems for TL, however at this stage PLGA has been the only polymer to have been studied extensively.