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TNF-receptor superfamily agonists as molecular adjuvants for cancer vaccines

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Abstract

Cancer vaccines have offered unrequited hope as a mechanism for rapidly and potently eliciting a patient's immune system to counter tumors. Initial results from preclinical mouse models have not translated to substantial benefit to patients, suggesting that either the targets or the vaccination approach were inadequate. Recent innovations in antigen identification have spiked renewed interest vaccination technologies. This has coincided with a detailed molecular understanding of the coordinated steps in post-activation support of T cell proliferation, differentiation and survival, leading to the development of novel targets and combinations that are substantially more effective than first and second generation cancer vaccines in preclinical models. Within this cluster of developments, the TNF-receptor superfamily members have emerged as attractive candidates for clinical implementation. Here we review recent developments in the mechanisms of action of TNFRSF agonists, and how their activity is potentiated by integration co-targeting pattern recognition receptors.

1. Introduction

While the capacity of the immune system to recognize and respond to tumors has been established for a long time, recent advances in understanding the checkpoint molecules by which tumor cells evade immune responses have led to unprecedented improvement in patient outcome in a variety of malignant settings [1]. However, while these advances are encouraging, the proportion of patients that receive benefit from these approaches remain limited. In part this is due to the absence of T cells within the tumor that can be target by checkpoint inhibitors. Thus, approaches that will increase the frequency of T cells within tumors are being considered as precursors for T cell-devoid tumors.

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2. Tumor vaccines

Peptide vaccines provide a opportunity to “heat up” tumors

Increasing the frequency of effector lymphocytes approaches include transgenic modification of T cells [2;3] and deployment of irradiation and other cancer-killing modalities to provoke inflammation within the tumor microenvironment. However, a particularly appealing approach is to exploit the antigenic repertoire of tumors by way of vaccination. Sustained attempts have been made to target families of “shared” antigens: tissue-specific antigens such as melanocyte-differentiation proteins; cancer-testis-differentiation antigens such as NYESO; and tumor-associated antigens such as E7 from HPV and KRAS mutations. However, in general the efficacy of this approach in patients has not lived up to the preclinical data generated in murine models, leading some to question its utility [4]. Although these self-antigens have generally proven to be immunogenic [5;6], some have argued that the relative sparseness of objective responses can in part be explained be the “self” nature of many of these targets (with the viral oncogenes being the exception). Indeed, the recent development of neo-antigen identification, in which the mutational repertoire of a patient’s tumor is scrutinized for immunogenic epitopes, has been enabled by advances in whole exome sequencing and pipeline bioinformatics, and may provide an individualized repertoire of antigens to target via vaccination [7;8].

Inadequacies of current vaccine adjuvants for peptide vaccines

An alternative explanation is that the current vaccination approaches are commonly modelled on adjuvants such as alum and montanide that were developed for the generation of protective antibody responses. While it is generally accepted that adaptive immune responses in the form of cytotoxic CD8⁺ and helper CD4⁺ T cells will be required for acute and long-term control of tumors, cancer patients provide unique scenarios for vaccine development. In cases where surgical resection to “no-evident disease” has occurred, prophylactic vaccines will likely be to generate protective memory, perhaps tissue-resident [9;10], where radiographically-undetectable micro-metastases reside. In others, such as widespread metastatic disease, large numbers of acute effector T cells with tissue homing properties will be necessary. Thus, adjuvants used for these diverse approaches could be quite distinct – what is clear, however, is we need to make considerable advances in the development of T cell “piloting” molecular adjuvants to augment immunity in a patient-context specific manner. Reinforcing this notion are recent studies from the Overwijk group demonstrating that the localized inflammation generated by adjuvants that are used as part of current vaccination protocols in clinical regimens can serve as a “black hole” for vaccine-driven T cells [11]. In unpublished data, we have also found that vaccine-specific T cells can be found circulating in breast cancer patients, but not in the target tumor. Thus, it is likely that improvements in the expansion, trafficking, retention and survival will be needed to increase the efficacy of vaccines. In aid of this, basic research has identified a toolbox for preclinical “vaccineers” to test and develop: cytokines, costimulatory molecules and checkpoint molecules, each of which can contribute to fate-decisions by T cells.

3. Initiating the CD8⁺ T cell response

Targeting innate sensors to promote vaccine efficacy

In the attempt to augment the effectiveness of peptide vaccines considerable efforts have been invested in understanding the mechanisms by which pathogens elicit CD8⁺ T cell responses. Convergent studies identified that the combined action of CD40 and toll-like receptor (TLR) stimulation lead to the potent activation of dendritic cells (DC), which in turn provide the costimulatory molecules and cytokines that contribute to the full-expansion and differentiation of CD8⁺ T cells. However, as CD40 stimulation is not always needed for the expansion of CD8⁺ T cells in response to highly inflammatory infectious agents, it was reasoned that the innate-stimulating ligands (Pathogen Associated Molecular Patterns; PAMPs) that are recognized by TLR and other pattern recognition molecules (PRR) might suffice to drive CD8⁺ T cell responses to peptide/protein vaccines. While the use of TLR agonists has been shown to be modestly effective in this capacity in murine therapeutic vaccination studies [12] few studies have shown that TLR ligands are sufficient to drive tumor-eradicating T cell responses. In part, this may be due to the consequences of systemic inflammation (e.g loss of chemokine-driven trafficking; inflammation induced desensitization of T cells; or even the ability of TLR stimulation to mobilize tumor cell metastases [13] that arises from administration of TLR agonists. Alternatively, it may not be possible to achieve, without sophisticated targeted deliver, the localized density of TLR-ligand that arises during an infection without accompanying systemic toxicity. In addition to TLR agonists, other PRR have been described, including members of the NOD-like receptors (NLR); C-type lectin receptors (CLR); and RIG-1-like receptors (RLR). Ligands for these molecules are being extensively studied for their ability to promote overall immunity in cancer, and some have examined their ability to serve as adjuvants for therapeutic vaccines [•14] but concerns exist about their relative ability to promote CD8⁺ T cell responses compared to mobilizing anti-inflammatory responses [15].

CD40 agonists potently expand peptide/protein vaccines

An alternative explanation for the modest efficacy of PRR agonists is that they are unlikely to engage the CD40-pathway of DC activation. Precursor naive CD4⁺ T cells are at too low frequency to drive extensive DC activation in response to de novo antigens (in the form of protein vaccinations), so mechanisms of targeting CD40 are full of potential. Pertinently, α GalCer has been shown to drive an NKT-mediated licensing of DC [16], and thus is being considered as an adjuvant that indirectly generates CD40 stimulation, and can perhaps in combination with TLR agonists [•17]. More commonly, agonistic antibodies to CD40 have been used to replicate CD40L-stimulation with DC to provoke CD8⁺ T cell responses to cancer vaccines [18]. Notably, in the absence of co-stimulating PRR [19] or TCR [20], or stimulation prior to T cell receptor engagement ([••21]; our unpublished data), CD40 stimulation can have deleterious effects on CD8⁺ T cell responses. Conversely, the combination of TLR stimulation and CD40 stimulation has become the gold-standard in protein-based cancer vaccines in pre-clinical models, often generating a response where 20–40% of the CD8⁺ T cells in the immunized animal being specific for the vaccinating antigen, and targeting CD40/TLR in combination with vaccination has been successful in control established tumors in multiple mouse [11;22;••23]. In these studies, and in our experience,

vaccination with anti-CD40 and peptide in the context of a TLR agonist is sufficient to drive large CD8⁺ T cell populations into tumors that were hitherto poorly infiltrated with T cells.

Why is CD40 stimulation of DC so potent? For one, systemic delivery of an agonistic antibody can activate DC in both secondary lymphoid tissue and potentially those that reside in tissues. Second, it promotes the expression of components of the antigen processing pathway, driving more effective generation of the peptide epitopes derived from the antigenic component of vaccines [24]. This is important for vaccines that consist of nucleic acid or protein, but perhaps less so for peptide-based vaccines. Third, it induces the expression of MHC class I and class II molecules and costimulatory molecules resulting in an increased density of peptide displayed on the DC surface in an immunogenic context [25]. Fourth, activated DC can produce chemokines that attract naive T cells which increases the chances of a productive engagement [26]. Fifth, it increases the expression of anti-apoptotic molecules, extending the viability and thus antigen presenting capabilities of DC [27]. Finally, stimulation of CD40 drives the expression of cytokines such as IL-12 and TNF-superfamily members that promote the activation and expansion and differentiation of T cells [28].

Clinical development of CD40 agonists has been slow, in part due to the existence of CD40 “sinks” in humans, and in part due to some dose-limiting toxicity that became evident in early phase trials [29], leading investigators to determine whether downstream effects of CD40 stimulation on DC can be exploited for vaccine purposes. Thus, attention has begun to focus on the TNF receptor superfamily (TNFRSF) members that are induced by CD40 stimulation, within which there potentially lies a treasure trove of opportunities to define even more finely sculpted subunit vaccine approaches due to their influence on elements of T cell fate decisions.

3. Modulation of T cell functions by agonistic TNFR-superfamily members

The TNFR-superfamily is composed of multiple members, subdivided into those that are associated with death domains (e.g.), non-signaling decoy receptors, and TRAF family E3 ubiquitin ligase-utilizing [30], generally costimulatory molecules (CD27; CD30; CD40; 41BB; OX40; GITR; HVEM; DR3; LT β R; TNFR1; TNFR2). The distribution and function of these molecules have been excellently reviewed recently [31;32]. For T cell-vaccine adjuvant purposes, most of the attention has focused on the TRAF-associated group, though it should be noted that exuberant T cell responses can elicit fratricide via TNFSF members, and relatively unexplored opportunities to promote the persistence of vaccine-induced T cells reside in this space. Within the costimulatory group, function is commonly associated with the promotion of T cell differentiation and survival, and to a lesser extent cellular proliferation. CD27, GITR and HVEM are expressed on naive T cells, while the remaining family members are induced after T cell activation. This temporal segregation between family members, in addition to their use of different TRAF adaptor molecules, suggests that the individual TNFSF receptors contribute to different facets of T cell responses. However, the timing and functional consequences of their individual vs concerted activation have not been carefully dissected. A general consensus about how they can contribute to the efficacy of cancer vaccines has, however, arisen.

Promotion of T cell survival

The majority of studies that have assessed the contribution of agonistic TNFR-superfamily members at the genetic level have been performed in the context of pathogenic infections. In the absence of TNFR-superfamily expression on the responding T cell, lesions in the magnitude of either primary, or more commonly, memory T cells are common. These effects are attributed to several consequences of TNFRSF signaling: the expression of transcription factors, such as Eomesodermin, that are consistent with the development of effector T cells with memory potential [17;•33–35] increased expression of anti-apoptotic molecules such as BcXL and reduced expression of BIM and Bax; limiting the expression of death receptors [36]; and the upregulation of the expression of common gamma chain cytokine receptors including IL-2 and the expression of IL-2 itself [•37–40]. While each of these elements undoubtedly contributes to the overall health and quality of a vaccine-driven T cell response, it's currently unclear whether they operate in serial or in parallel.

Attenuation of regulatory T cells (Treg)

Regulatory T cells represent a barrier to effector T cell activation and function, and constrain T cell responses driven by either tumor antigen or vaccination. TNF-superfamily members are commonly constitutively expressed on Treg, often at levels higher than on conventional CD4, CD8 and NK cells, and it has been argued that tonic TNFSF signaling is necessary for Treg persistence [41;42]. Antibodies specific for these TNFSF/-R members commonly result in reductions in Treg numbers or function [40;43–••46]. Whether this effect is a function of antibody-dependent cytotoxicity; blocking receptor-ligand interactions; or an increase in signal intensity driving activation-induced cell death remains to be discerned, as does the relative contribution of Treg ablation to the effectiveness of TNFSFR-targeting antibodies in driving effector T cell responses.

4. Promoting TNFSFR responses

Inclusion of inflammation

Much initial exploration of the efficacy of TNFRSF-targeting antibodies has been performed in the context of pathogenic infections that are inherently inflammatory. Of note, induction of TNFSFR expression is generally insufficient to over-ride the requirement for CD28-mediated costimulation [19;47] though acute inflammation can cooperate with CD27 stimulation to initiate T cell priming [48]. Together these data suggest that the TRAF/JNK/NFκB pathway activated by TNFSFR stimulation is inadequate to drive T cell cycling, and that the signals provided by CD28 or inflammatory mediators in combination may be necessary to either “authorize” TNFSFR signaling, or work in parallel with it. Pertaining to this, although some studies have indicated that these antibodies are effective at raising T cell responses when used as part of subunit vaccination approaches, in many of these instances endotoxin was not removed from the antibody or antigenic component of the vaccines. This is a situation that will not be permissible clinically, and studies by several labs have shown that endotoxin-free antibodies against TNFRSF members have limited activity in vaccination settings [35;49]. This raises two important questions. First, how is endotoxin promoting the activity of these antibodies, and second, what inflammatory components are best paired with TNFSFR-targeting approaches to safely mimic/improve upon endotoxin? Targeting either

CD27 or 4-1BB can promote Eomes expression [17;35] yet the induction of T-bet, a transcription factor that substantially contributes to effector T cell development, required type-1 interferons. Type-1 interferons have also been shown to protect nascently-activated T cells from cytolysis by NK cells [50]. Type-1 interferon, or the elaboration of pro-inflammatory cytokines including IL-6, IL-12 or IL-27 [51], may also provide indirect support to expanding T cell responses, in the form of APC activation and attenuation of Treg function. Some studies have assessed the relative ability of different PRR agonists to work in conjunction with different TNFRSF [52], or whether PRR ligands should be conjugated to agonistic antibodies [53] but few have assessed whether such interactivity can influence vaccine-generated T cell-mediated control of established tumors.

FcR engagement/antibody multimerization

While antibodies that block checkpoint inhibitory molecules have shown exciting efficacy in clinical trials, early phase studies with agonistic antibodies targeting TNFRSF members have shown evidence of biological activity (T cell activation markers are commonly induced, along with T cell cycling), they have not shown consistent impact on patient disease status. In part this may be due to remaining mechanisms of adaptive resistance within the tumor microenvironment. However, recent studies have revealed a hitherto under-appreciated role for cross-linking TNFRSF for full activation [54–56], perhaps a consequence of the natural trimeric-state of many TNFSF ligands. In vivo, cross-linking is most commonly achieved by antibody Fc regions binding to Fc receptors (FcR) on antigen presenting cells, such as monocytes or B cells. Indeed, in our hands the activity of either CD40 or CD27 stimulatory antibodies at eliciting T cell responses in the context of vaccination is abrogated in the absence of B cells or DC (McClintic et al; manuscript in preparation). In vitro screening of TNFRSF antibodies can often suggest that they have antagonistic activity unless they are either plastic-bound or presented by APC. Thus agonistic antibodies may have reduced activity in environments that have limited FcR availability, and approaches that are designed to curtail immature myeloid cells may negatively impact the in vivo cross-linking of antibodies. Importantly, data suggest that a hierarchy exists with respect to the effectiveness of cross-linking by different FcR, with Fc γ R IIB being most active and Fc γ RIIA being inhibitory [57]. This suggests that careful consideration of the framework of TNFRSF-targeting antibodies will need to be considered, with IgG1 subtypes perhaps being most efficacious [58] for cross-linking and impeding regulatory T cells, and raises the possibility that trimeric ligands may be a more optimal approach for stimulating TNFRSF in vivo. IgG1 molecules may, of course, lead to deletion of other subsets of cells expressing the targeting TNFSFR, and overt removal of Treg in combination with systemic inflammation may reveal unanticipated toxicities.

Timing of delivery

While it is established that certain TNFSFR members are constitutively available and others induced as T cells progress from their naive to fully activated state, the impact of the timing of stimulation, or the chronicity of stimulation, needs to be considered. Chronic expression of CD70 can lead to excessive differentiation and activation-induced cell death of CD27-expressing T cells in murine models, and stimulation of TNFSRF during chronic viral infections can attenuate T cell responses [59]. To date, sustained agonistic CD27 and OX40

antibodies have not been reported to cause lympho-depletion as monotherapies, though this will need to be monitored as vaccine-based trials are begun, yet high doses of 4-1BB agonistic antibody led to hepatotoxicity [60]. Pertaining to this, remarkable little is known about the expression of TNFSF members during the contraction phase of T cell responses and ongoing contribution of these molecules in sustaining memory cells [61]. These need to be considered and will influence the tempo and sequencing of TNFRSF stimulation during vaccination protocols.

5. Ongoing questions

While agonistic antibodies to the majority of the TNFR-superfamily have been developed for both murine and human targets, their use in support of vaccines in cancer is only just beginning to be explored clinically. Aside from defining optimal combinations with PRR and the timing of TNFRSF engagement, very little is known about whether and how different TNFRSF contribute to T cell fate decisions, nor the mechanistic basis of the synergy with PRR. Some studies have indicated that CD27 might drive more proliferative, effector responses, while 4-1BB promotes memory [62]. An elegant assessment of how 4-1BB signaling influences CAR-T differentiation has confirmed the concept that 4-1BB might promote memory [63], perhaps by contributing to metabolic fate decisions that promote fatty acid oxidation [64]. This kind of information may help guide which TNFRSF member should be targeted in therapeutic or prophylactic settings. Interestingly, preliminary data suggest that sequencing of TNFRSF stimulation can also have a profound influence on the quality and quantity of T cell responses, and should be considered in the construction of vaccination protocols (Jeong et al; manuscript in preparation). While these data support the notion that different TNFRSF have different responsibilities in programming T cell fate-decisions, how this occurs and whether different agonists can be selected to sculpt the qualities of the primary and memory T cell populations is now under investigation, along with their combination with inhibition of CTLA-4, PD-1 and other checkpoint molecules. Of particular interest will be defining whether different TNFRSF are responsible for promoting/sustaining central, effector and tissue-resident memory T cell populations, and whether similar outcomes are found with MHC class II/CD4⁺ T cell target vaccinations as found with MHC class I/CD8⁺ T cells. As a cautionary note, however, it can be difficult to assign “functions” to different TNFRSF members due to differences in antibody quality, whether FcR-mediated (as discussed above) or due to the affinity or specificity of the Fab domains. Thus, it will be important to consider whether different antibody clones drive similar outcomes in T cell biology. Finally, the potential contributions of targeting CD30 and HVEM have received very little attention with respect to vaccine development.

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Highlights

- CD40-mediated induction of TNFSF ligands on APC, or targeting TNFRSF receptors represents a 3rd generation vaccination approach with exponentially larger T cell responses than previously achieved.
- PRR-induced inflammatory cytokines synergize with stimulation of TNFSF receptors by activating nodes of transcription.
- The efficacy of targeting TNFRSF may be augmented by a concurrent reduction in regulatory T cell frequency and function.
- FcR-mediated cross-linking dynamically influences the activity of TNFRSF antibodies suggesting that design-build approaches to targeting TNFRSF members could lead to even more potent agonists.

Figure 1. Synergistic mechanisms that support T cell expansion and activity in cancer vaccines when targeting TNFRSF

The combination of cross-linking antibodies specific for TNFRSF members and innate cytokines, such as IFN-1, driven by engagement of PRR, synergize to expand conventional effector T cells (Tcon). Additional activity against regulatory T cells (Treg) likely compound the efficacy of targeting this pathway. Many members of the TNFRSF are inducible, while others are constitutively expressed by both Tcon and Treg. Nodes of metabolic activity and survival likely contribute to T cell fate decisions that occur after TNFRSF engagement, and could be exploited to sculpt qualities of T cell responses for different elements of cancer vaccine implementation, affecting efficacy.

Table 1

Pre-clinical studies of TNFRSF stimulation and cancer vaccines

TNFRSF-member	Immunogen	Combination	Context	Reference
OX40	MHC class II peptides/CD4 T cells	TLR agonists: LPS; CpG; PolyIC; GDQ	Melanoma	[23]
	Peptide Dribbles/DriPS/SLiPS		Breast cancer	[65]
	Glioma Tumor lysate; compared to GITR-L and 4-1BBL	CpG/poly IC	Glioma	[66]
GITR	Peptide	anti-PD1	Melanoma	[67]
	Protein pulsed bone marrow adherent cells		Lung cancer	[68]
CD27	Tumor lysate+DC vaccine		Prostate cancer	[69]
	E7-DNA vaccine	anti-PD1	Cervical cancer	[70]
	Protein vaccination; compared to 4-1BB		Immunological memory	[62]
	Protein vaccination; vaccinia protection	polyIC	Viral therapy	[35]
41BB	E7 Peptide vaccine	α GalCer;	Cervical cancer	[17]
	Attenuated vaccinia and peptide	IFA	Viral therapy	[71]
	TRP-2 peptide	CpG	Melanoma	[72]
	E7-DNA vaccine	IL-2/IL-15	Cervical cancer	[73]
	E7 peptide	MLA	Cervical cancer	[74]