Seminars in Immunology 25 (2013) 182–190

Contents lists available at SciVerse ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Review

Prospects of combinatorial synthetic peptide vaccine-based immunotherapy against cancer

Ramon Arens[a,*], Thorbald van Hall[b], Sjoerd H. van der Burg[b], Ferry Ossendorp[a], Cornelis J.M. Melief[a,c]

[a] Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands
[b] Department of Clinical Oncology, Leiden University Medical Center, Leiden, The Netherlands
[c] ISA Pharmaceuticals B.V., Leiden, The Netherlands

ARTICLE INFO

Keywords:
Animals
Cancer
Chemotherapy
Dendritic cells
Patients
T-cells
Vaccine

ABSTRACT

The insight that the immune system is involved in tumor resistance is gaining momentum and this has led to the development of immunotherapeutic strategies aiming at enhancement of immune-mediated tumor destruction. Although some of these strategies have moderate clinical benefit, most stand-alone therapies fail to significantly affect progressive disease and survival or do so only in a minority of patients. Research on the mechanisms underlying the generation of immune responses against tumors and the immune evasion by tumors has emphasized that various mechanisms simultaneously prevent effective immunity against cancer including inefficient presentation of tumor antigens by dendritic cells and induction of negative immune regulation by regulatory T-cells (Tregs) and myeloid derived suppressor cells (MDSCs). Thus the design of therapies that simultaneously improve effective tumor immunity and counteract immune evasion by tumors seems most desirable for clinical efficacy. As it is unlikely that a single immunotherapeutic strategy addresses all necessary requirements, combinatorial strategies that act synergistically need to be developed. Here we discuss the current knowledge and prospects of treatment with synthetic peptide vaccines that stimulate tumor-specific T-cell responses combined with adjuvants, immune modulating antibodies, cytokines and chemotherapy. We conclude that combinatorial approaches have the best potency to accomplish the most significant tumor destruction but further research is required to optimize such approaches.

1. Introduction

Despite the clinical success of conventional anti-cancer therapies (surgery, chemo- and radiotherapy) many cancers are not curable. Among the limitations of these therapies are the toxicity and the relatively low effectiveness of the treatment of metastatic cancer. However, based on new insights, novel biologic therapies have shown promising results. In depth studies of patients on experimental protocols together with informative studies of murine tumor models have unequivocally demonstrated that the immune system plays a major role in tumor outcome [1,2]. Moreover, most cancer patients mount immune reactions to developing neoplastic cells and a number of tumor-associated antigens that trigger T and B cell responses have been identified [3–5]. Together, these findings have led to the development of therapeutic strategies that aim to enhance immune-mediated tumor destruction and to counteract tumor-induced immune suppression.

Current immunotherapeutic approaches include therapeutic vaccines that elicit anti-tumor T-cell responses, monoclonal antibody therapies that activate the immune system directly or antagonize negative immune regulation, recombinant cytokines, and adoptive cellular therapies. While these manipulations increase tumor immunity, unfortunately most stand-alone therapies fail to significantly affect progressive disease or do so only in a minority of patients. Ongoing research on the mechanisms underlying the generation of anti-tumor responses and the immune evasion by tumors have underscored that multiple mechanisms together restrain the potency of host responses including inefficient presentation of tumor antigens by dendritic cells and induction of negative immune regulation such as the development of regulatory T-cells (Tregs) and myeloid derived suppressor cells (MDSCs) [6–8]. Thus the design of therapies that simultaneously improve effective tumor immunity and counteract immune evasion by the tumor seems most desirable for clinical efficacy. As it seems unlikely that a single therapy addresses all necessary requirements, combinatorial strategies that act synergistically need to be developed. This review discusses the development of combinatorial immunotherapies as

* Corresponding author at: Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands. Tel.: +31 715263174; fax: +31 715265267.
E-mail address: R.Arens@lumc.nl (R. Arens).

1044-5323/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.smim.2013.04.008

in our opinion such approaches have the potency to accomplish the most significant tumor destruction and control.

2. Synthetic peptide vaccines and adjuvants

The immune system has the ability to recognize and eliminate cancerous cells. In particular, CD8 cytotoxic T-cells (CTLs) that detect tumor antigens presented by MHC class I molecules on the surface of tumor cells have been shown to be potent effector cells for tumor eradication. Based on this principle, class I-binding synthetic peptides that stimulate tumor-specific CTLs have been developed as therapeutic vaccines against established tumors. Whereas earlier generations of synthetic peptide vaccines were composed of one to several class I-restricted peptides, newer generations of peptide vaccines consist of multivalent long peptides that incorporate both class I CTL and class II helper epitopes [9]. This development was driven by preclinical and clinical studies indicating that a number of parameters, such as the length of the peptide and CD4 T-cell help, can influence the safety and vaccine efficacy [10,11] even for MHC class II negative tumors [12]. The inclusion of multiple peptide epitopes to diversify the anti-tumor response can help to reduce tumor antigen-loss variants [13]. Furthermore it became apparent that inclusion of adjuvants is crucial as peptides by themselves (without adjuvants) are poorly immunogenic or can even induce tolerance.

Incomplete Freund's adjuvant (IFA) or the equivalent Montanide ISA-51 is water in oil emulsions that are commonly used in peptide vaccines as adjuvants. Their main function is likely depot formation that inhibits immediate systemic bio-distribution of the peptides and improves uptake by antigen-presenting cells (APCs). Short (9–11 amino acids) peptides in IFA or Montanide (without other agents) administered subcutaneously (s.c.) can elicit detectable CD8 T-cell responses against melanoma but clinical responses in melanoma patients are unsatisfactory [14–19]. This is likely related to dysfunction and deletion of specific T-cells at the vaccination site [20], indicating that in case of short peptide vaccines combinations with other adjuvants and/or other therapeutic strategies are required. Long peptide vaccines in IFA however do not result in T-cell dysfunction [20].

Long overlapping peptides, together covering the entire length of the oncogenic proteins E6 and E7 of high-risk human papilloma virus type 16 (HPV16) dissolved in Montanide are highly immunogenic [21,22]. Such peptide vaccines can cause partial or complete regression of premalignant lesions in the majority of women with HPV16-induced high-grade vulvar intraepithelial neoplasia [23]. The nature and strength of the vaccine-induced T-cell response correlated with the clinical response [23,24]. Nevertheless, this clinically active vaccine has not shown potent clinical activity in patients with HPV16-induced end-stage cancer [21] or recurrent cervical cancer (unpublished results) whereas it was able to evoke HPV16-specific T-cell responses. In our opinion, such vaccines promise to be of use to treat malignant tumors caused by this virus, including HPV16-induced cancers of the cervix, vulva, anus and head- and neck region when combined with stronger adjuvants, other immunotherapeutic approaches and/or chemotherapy, as we will discuss below.

Further evidence for clinical activity of the long peptide vaccine concept was obtained with a 16 amino acid long peptide used to vaccinate against telomerase reverse transcriptase (TERT), a universally overexpressed tumor antigen. This peptide, dissolved in Montanide, proved effective in eliciting specific T-cell immunity, and this was associated with prolonged survival in patients with advanced non-small-cell lung cancer [25,26]. Furthermore, a 14 amino acid long peptide vaccine (Rindopepimut) that elicits cellular and humoral responses against epidermal growth factor receptor variant III (EGFRvIII) demonstrated some (pre-)clinical efficacy. First in preclinical studies it was demonstrated that Rindopepimut induces efficient generation of EGFRvIII-specific humoral and cellular immune responses as well as lasting tumor regression and increased survival times [27]. Next, clinical trials in patients with glioblastoma demonstrated significantly increased median time to progression and overall survival time in those vaccinated with Rindopepimut compared with matched historical controls [28].

Unmethylated CpG motifs activate APCs via binding to TLR9 ligand [29]. Added to peptide vaccines, CpG markedly improves the anti-tumor T-cell response in experimental models and in humans [30–38]. CpG is able to improve the vaccine efficacy of both short and long peptides in IFA or Montanide but also of peptides in saline [36,39], indicating that CpG is a good adjuvant by itself but can also be used to strengthen depot forming adjuvants. For optimal adjuvant efficacy of CpG, however, it is important that CpG is maintained in close proximity to the released antigens [40–42].

Although the TLR3 ligands Poly-IC and modified Poly-IC (Poly-ICLC) have proven to be successful adjuvants in experimental models when combined with peptide vaccines [36,43–45], objective clinical benefit of these approaches awaits further study. Also TLR2 and TLR7 agonists can be efficacious with peptide vaccines [46–48], which provides supplementary combinatorial opportunities to adjuvant peptide vaccine-based approaches against cancer.

Based on the idea that co-delivery of antigen and adjuvant to the same location/cell (generally injected s.c.) would enhance vaccine immunogenicity, conjugation of TLR ligands with protein/peptide antigens was developed and functional studies indeed confirmed more potent adjuvant properties in both cancer models and pathogen-induced disease settings [49,50]. In depth studies showed that TLR ligand–peptide conjugates improve the trafficking and intracellular processing pathways leading to optimal antigen presentation and T-cell priming [48].

Summarizing, we conclude that TLR ligands are good adjuvants as inclusion of at least one of these compounds in synthetic peptide vaccines against tumors is important for induction of robust anti-tumor T-cell responses. Combination of some of these TLR ligands acts synergistically [51,52] and thus may further enhance vaccine adjuvant properties [53]. In addition, combinations with other immune stimulatory ligands like NOD ligands and RIG-I ligands could further enhance synthetic peptide vaccine moieties [54]. Furthermore, combining with strategies that block tumor-associated immunosuppression (such as those discussed hereafter) is likely essential for further enhancement of clinical benefit.

3. Synthetic peptide vaccines combined with other immunomodulatory compounds

3.1. Cytokines

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that is used as an adjuvant in vaccines due to its capacity to cause proliferation of dendritic cells and macrophages. In melanoma patients, the addition of locally applied GM-CSF modestly increased the immune response against peptide/IFA vaccines [55,56]. TERT peptide vaccines administered with GM-CSF in pancreatic and lung cancer patients induced detectable vaccine-induced T-cell responses and survival advantage in immune responders [57–59]. Likewise, TERT peptide vaccination in combination with GM-SCF in pancreatic cancer patients resulted in demonstrable T-cell responses [57]. Also synthetic peptides in Montanide and/or adjuvanted with CpG and delivered subcutaneously with GM-CSF elicited clearly measurable CD8 T-cell responses in cancer patients [19,60–62]. Patients with high vaccine-induced T-cell responses had a better overall survival than