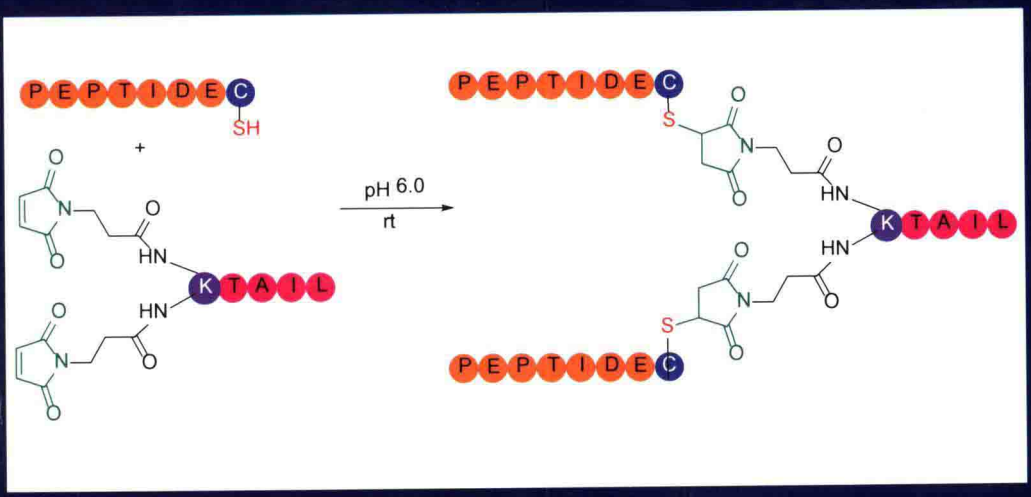
Advances in
PROTEIN CHEMISTRY and STRUCTURAL BIOLOGY

Peptide and Protein Vaccines



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Rossen Donev

VOLUME NINETY NINE

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ROSSEN DONEV

Biomed Consultd Ltd

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Peptide and Protein Vaccines

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PREFACE

Conventional vaccine strategies proved highly efficacious for decades in reducing mortality and morbidity due to infectious diseases. The major drawbacks of conventional vaccines, such as those including whole organisms or large proteins, seem to be the inclusion of unwanted antigens that not only contributes little to the protective immune response but also are likely to promote allergenic and/or reactogenic responses. Thus, peptide vaccines are an attractive alternative strategy based on the engineering of short peptide fragments that induce highly targeted immune responses, allowing the avoidance of allergenic and/or reactogenic reactions. A successful vaccine candidate should meet several criteria: immunogenicity, specificity, protective activity, and durability. First, it should be immunogenic, i.e., it should be able to elicit an immune response, both humoral and cell-mediated, that leads to the blocking and eradication of the disease-causing agent and clearance of the affected cells. Second, this immune response must specifically target (bind with a certain affinity) the region on the pathogen that has been mimicked by the immunogen used as a vaccine, that being a peptide, a protein, or a whole organism, and it must avoid cross-reaction with other self-antigens to prevent autoimmunity. Third, the elicited immune response should be able to prevent the establishment of a disease if the aim is to pursue a prophylactic vaccine, which is usually the case in pathogen-mediated infections, including prions. Finally, the vaccine must be able to induce the production of B and T memory cells, both required to ensure re-elicitation of the protective immune response should the organism encounter the pathogenic agent even years after immunization (Apellániz & Nieva, 2015).

A peptide vaccine usually consists of one or more peptide sequences of more than 15 amino acids long that induce B and T cell stimulation when presented by itself or bound to carrier proteins, scaffolds, or supramolecular complexes (e.g., liposomes). Moreover, peptides from one or several strains of the same pathogen might be included to ensure proper coverage of pathogen variability. Some peptide-based formulations devised following these strategies have rendered vaccines effective in preventing viral infection in animals (Bittle et al., 1982; Langeveld et al., 1994).

First chapter in this volume discusses new promising strategies of peptide vaccine development recently progressed in preclinical and/or clinical stage with main focus on the roles of peptides in the vaccine formulation from

epitope to adjuvant. Second article in this thematic volume gives an overview of applications of lipid vesicles (liposomes) to the development of membrane-proximal external region-targeting vaccines, both as type B adjuvants and epitope structure-shaping devices. This chapter introduces a new paradigm in peptide vaccine development: the structural stabilization of peptide epitopes through contacts with the membrane surface. The third chapter in this volume discusses strategies for developing successful prophylactic and therapeutic vaccines using lipoprotein-based immunogens that are safe, cost-effective, and suitable for human use. Authors support their point of view with a number of examples that demonstrate the merit of lipoproteins with intrinsic adjuvant properties for novel vaccine development. In the fourth chapter, authors review a strategy for improving the efficacy of peptide vaccines. They discuss recent studies providing a potent method of epitope screening and antibody production without conventional carriers. Instead, they adopted Lipoplex(O), comprising a natural phosphodiester bond CpG-DNA and a specific liposome complex, as an adjuvant. Lipoplex(O) induces potent stimulatory activity in humans and mice, and immunization of mice with several peptides co-encapsulated with Lipoplex(O) without carriers significantly induces each peptide-specific IgG2a production. This strategy can be applied in development of therapeutic antibodies or in defense against pandemic infectious diseases through rapid screening of potent B cell epitopes. In the fifth article of the thematic volume, authors discuss several different chemistries that have been pursued to obtain novel platforms onto which antigenic epitopes can be tethered, with the aim to achieve a higher antibody response. In this regard, they review the chemical strategies developed for the presentation of peptide epitopes. The final sixth chapter in this volume focuses on the role of mutations in viral proteins for the design strategy of vaccines against the viruses, which has been exemplified in hepatitis B virus.

The aim of this volume is to promote further research and development in the design of peptide vaccines in order to achieve highly targeted immune responses against different pathological conditions while avoiding allergenic and/or reactogenic reactions.

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CONTENTS

CHAPTER ONE

Peptide Immunotherapy in Vaccine Development: From Epitope to Adjuvant

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Contents

Abstract

Vaccines are designed to educate the host immune system to prevent infectious disease or to fight against various diseases such as cancers. Peptides were first employed to provide specific immune responses while minimizing unintended allergenic or reactogenic adverse effects. Discoveries of virus or cancer-specific antigens and the advanced knowledge of immunology accelerate the peptide vaccine development. Despite the overwhelming research pipelines, a very few of them reached to market approvals or phase III clinical trials, because of the lack of efficacy. Several strategies for the next generation peptide vaccines are devised to overcome the weak immunogenicity and the poor delivery. In this review, we discuss the new promising strategies of peptide vaccine development which are recently developed in preclinical and/or clinical stage focusing the roles of peptides in the vaccine formulation from epitope to adjuvant. Additionally, we discuss the future perspectives of peptide vaccine and immunotherapy.

1. INTRODUCTION

Synthetic peptide vaccines are usually composed of 20–30 amino acids containing the specific epitope of an antigen related to infectious and/or chronic diseases including cancers. Peptide vaccines theoretically have several advantages over other types of vaccines such as conventional vaccines

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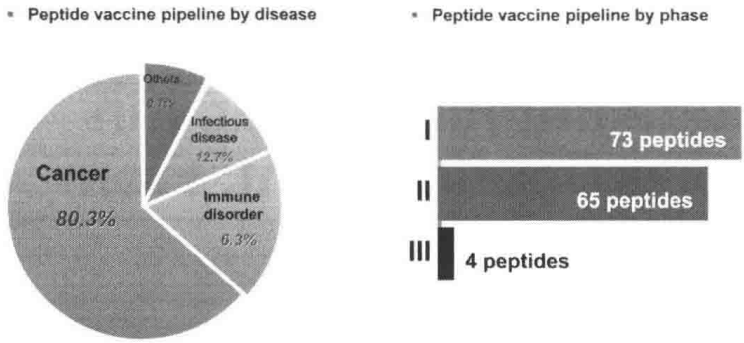

Figure 1 Current development of peptide vaccine (2015).

and newly developed DNA or cellular vaccines (Ingolotti, Kawalekar, Shedlock, Muthumani, & Weiner, 2010). Easy synthesis with low cost, increased stability, and relative safety are generally demonstrated in numerous preclinical and clinical studies. In addition, peptide vaccines have no limitation in target diseases from virus infection to Alzheimer disease and even allergy (Fig. 1A) (Larche, 2007; Mocellin, Pilati, & Nitti, 2009; Nava-Parada, Forni, Knutson, Pease, & Celis, 2007; Park et al., 2014). Peptide vaccines can be designed with self- or nonself-antigen to properly balance the immune responses, which is not possible for conventional vaccines (Purcell, McCluskey, & Rossjohn, 2007). However, it was recently reported that no peptide vaccine is approved by FDA, although more than 500 peptides had progressed to clinical trials (Li, Joshi, Singhania, Ramsey, & Murthy, 2014). According to ClinicalTrials.gov, a public database which is a service of the U.S. National Institute of Health, there are 73 clinical trials in phase I, 65 clinical trials in phase II, and 4 clinical trials in phase III in the search result of peptide vaccine in March 2015 (Fig. 1B). The series of failures in peptide vaccines in clinical trials suggest several issues critical for the successful development of peptide vaccines. These include (1) limitation of single peptide epitopes as vaccine candidates, (2) an immune evasion, (3) the failure to elicit the controlled and prolonged immune response, (4) a lack of efficacy, and (5) the inappropriate design of clinical trials. In this review, we discuss the roles of peptides in vaccine formulation focusing on the innovative approach overcoming those limitations in the recent clinical studies or the researches close to clinical development.

2. CANCER VACCINE

The rational of cancer vaccine development is that tumor cells can be eradicated by induction of cytotoxic T lymphocyte (CTL) response against

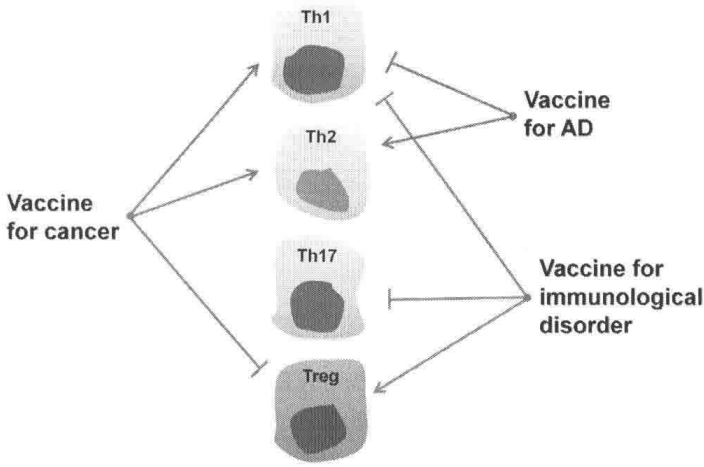

Figure 2 Rational design of peptide vaccines for specific diseases.

tumor-associated antigen (TAA) (Fig. 2) (Inoda et al., 2011; Melief & van der Burg, 2008). TAAs are generally processed in antigen-presenting cells (APCs) and presented to T cells in a human leukocyte antigen (HLA)-restricted pathway (Hirano et al., 2006; Parmiani et al., 2002). TAA-specific CTL can be activated and attack cancer cells recognized by TAA expression (Parmiani et al., 2002). Many peptide cancer vaccines are designed to stimulate T cells but most of them failed to show clinical benefits in clinical trials even though some of the vaccines successfully activated APC and TAA-specific T cells. The lack of vaccine efficacy was thought to result from HLA restriction, diversity of cancer phenotype, and immune evasion (Chentoufi et al., 2010; Khong & Restifo, 2002). To overcome these limitations, multiple peptides are employed in the development of cancer vaccine formulation rather than a single peptide.

IMA901 (Immatics) is a peptide cancer vaccine composed of multiple tumor-associated peptides (TUMAPs) using GM-CSF as adjuvant (Walter, Weinschenk, Reinhardt, & Singh-Jasuja, 2013; Walter et al., 2012). TUMAPs consist of 10 different peptide epitopes (Fig. 3), which are found to be overexpressed in the majority of renal cell carcinoma (RCC) (Bedke & Stenzl, 2013; Rausch, Kruck, Stenzl, & Bedke, 2014).

In a phase II clinical trial of IMA901, a randomized trial with 68 HLA-A*02-positive RCC patients, the group 1 patients (n=35) received IMA901 while the group 2 patients (n=33) received IMA901 with the pretreatment of cyclophosphamide (300 mg/m, n=35) or IMA901 preceded by a single immune modulatory dose of i.v. cyclophosphamide (group 2 [n=33]; 300 mg/m^2) (Pal, Hu, & Figlin, 2013; Walter et al., 2012). The patient

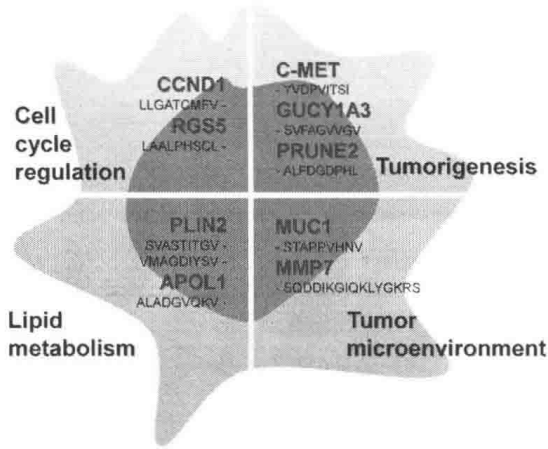

Figure 3 Tumor-associated peptides (TUMAPs) in IMA901 cancer vaccine.

groups then received up to 17 injections of IMA901 plus GM-CSF over a period of up to 9 months (Walter et al., 2012). Among those patients that elicited an immune response to IMA901, cyclophosphamide pretreatment was associated with a significantly prolonged survival (Rausch et al., 2014; Walter et al., 2012). However, cyclophosphamide pretreatment had no impact on survival in the subset of patients who lacked an immune response. IMA901 is currently in a phase III clinical trial and has been granted orphan drug designation in the United States and Europe for the treatment RCC in HLA-A*02-positive patients.

On the other hand, GV1001 (KAEL-GemVax) is a cancer vaccine composed of a single synthetic peptide containing multiple epitopes in a 16-amino acid-long peptide. The peptide is derived from the human telomerase reverse transcriptase (Brunsvig et al., 2006; Park et al., 2014). Because GV1001 could bind to multiple HLA class I and HLA class II molecules (Kyte, 2009), it may therefore elicit combined CD4/CD8 T cell response, considered important to initiate tumor eradication and also long-term memory (Raval, Sharabi, Walker, Drake, & Sharma, 2014). In a phase II study with advanced pancreatic cancer patients, GV1001 showed total immune responses (63%) and a greater median survival (216 days; 146–323) in immune responders than in nonresponders (88 days; 53–190) (Bernhardt et al., 2006; Middleton et al., 2014). However, in a three-group, open-label, randomized phase III trial with locally advanced or metastatic pancreatic cancer patients, GV1001 fails to improve overall survival. Interestingly, cytokine analysis for the prediction of biomarker revealed that in the subset of patients with high eotaxin (CLL11) level GV1001 improved

the median overall survival (high eotaxin = 14.8 vs. low eotaxin = 7.9) (Neoptolemos, 2014). Although the efficacy of GV1001 depending on eotaxin levels should be examined in a large phase III clinical trial, GV1001 recently received new drug approval in Korea for the pancreatic cancer patients with high level of eotaxin.

3. ALLERGY VACCINE

In normal physiological condition, body's immune system operates in steady-state condition to maintain immune homeostasis (Liu et al., 2007). However, abnormal failure of this homeostasis results in immune disorders such as autoimmune diseases and allergies (Burton & Oettgen, 2011). As we more understand the immunopathological events in immune-related diseases through the series of clinical studies, many innovative immunotherapies have been designed including vaccines.

Cat-Pad (Circassia) is a cat allergy vaccine composed of seven synthetic peptide immunogen-regulatory epitopes originated from the major cat allergen Fel d 1 (Worm, Patel, & Creticos, 2013). Because native allergen or long peptides can induce allergenic responses, Cat-Pad employed seven short peptides (13–17 amino acids) derived from binding analysis to common HLA-DR molecules (Table 1) which enable the peptides to bind to broad range of HLA molecules (Worm et al., 2013). In the randomized, double-blind, placebo-controlled study (phase IIb), the vaccine was well tolerated and demonstrated clinical benefits in both Total Rhinoconjunctivitis

Table 1 Fel d 1 Peptide Sequences in Cat-Pad, a Cat Allergy Vaccine

Peptide Origin	Length	Epitope	Sequence
Fel d 1 chain 1 (3–15)	13	DR3,15	CPAVKRDVDLFLT
Fel d 1 chain 1 (23–38)	16	DR1,4,11,13,15	EQVAQYKALPVVLENA
Fel d 1 chain 1 (29–45)	17	DR1,3,4,11,13,15 DRB4,5	KALPVVLENARILNCV
Fel d 1 chain 1 (39–55)	17	DR3,4	RILKNCVDAKMTEEDKE
Fel d 1 chain 1 (54–69)	16	DR11,13,15	KENALSLLDKIYTSPL
Fel d 1 chain 2 (40–55)	16	DR15 DRB4	TAMKKIQDCYVENGLI
Fel d 1 chain 2 (56–71)	16	DR4,11 DRB4	SRVLDGLVMTTISSSK