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Research review paper

Epitope-driven DNA vaccine design employing immunoinformatics against B-cell lymphoma: A biotech's challenge

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ABSTRACT

DNA vaccination has been widely explored to develop new, alternative and efficient vaccines for cancer immunotherapy.
DNA vaccines offer several benefits such as specific targeting, use of multiple genes to enhance immunity and reduced risk compared to conventional vaccines.
Rapid developments in molecular biology and immunoinformatics enable rational design approaches. These technologies allow construction of DNA vaccines encoding selected tumor antigens together with molecules to direct and amplify the desired effector pathways, as well as highly targeted vaccines aimed at specific epitopes.
Reliable predictions of immunogenic T cell epitope peptides are crucial for rational vaccine design and represent a key problem in immunoinformatics. Computational approaches have been developed to facilitate the process of epitope detection and show potential applications to the immunotherapeutic treatment of cancer. In this review a number of different epitope prediction methods are briefly illustrated and effective use of these resources to support experimental studies is described.
Epitope-driven vaccine design employs these bioinformatics algorithms to identify potential targets of vaccines against cancer.
In this paper the selection of T cell epitopes to develop epitope-based vaccines, the need for $CD4^+$ T cell help for improved vaccines and the assessment of vaccine performance against tumor are reviewed.
We focused on two applications, namely prediction of novel T cell epitopes and epitope enhancement by sequence modification, and combined rationale design with bioinformatics for creation of new synthetic mini-genes.
This review describes the development of epitope-based DNA vaccines and their antitumor effects in preclinical research against B-cell lymphoma, corroborating the usefulness of this platform as a potential tool for cancer therapy.
Achievements in the field of DNA vaccines allow to overcome hurdles to clinical translation. In a scenario where the vaccine industry is rapidly changing from a mostly empirical approach to a rational design approach, these new technologies promise to discover and develop high-value vaccines, creating a new opportunity for future markets.

Contents

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1. Introduction

DNA vaccines offer a novel class of drugs with great therapeutic potential as innovative approach for cancer immunotherapy. The development of nucleic acid-based therapeutics has raised great interest in the past two decades as a new category of biotechnology product for highly specific medical interventions at the molecular scale. Plasmid DNA vectors offer the potential to develop potent vaccines and novel therapeutics to cure many diseases, from viral, bacterial, or parasitic infections to hereditary diseases and cancer. Starting with the discovery that the injection of a DNA plasmid in mouse muscle has resulted in a significant expression of the protein encoded by the plasmid (Wolff et al., 1990), various antigens have been successfully used to induce the production of antibodies (Abs) and cytotoxic T lymphocytes (CTLs) (Cox et al., 1993; Roy et al., 2000; Tang et al., 1992; Ulmer et al., 1993; Ulmer et al., 1996), thereby demonstrating the potential of this strategy for DNA vaccination. Progress in this field has resulted in the development and the marketing of four veterinary DNA vaccines (Liu, 2010).

DNA vaccines simply use plasmid DNA, which contains a DNA sequence coding for an antigen (Ag) and a promoter for gene expression in the mammalian cell. Plasmid DNA does not require formulation or viral vector for delivery. Naked DNA is safe and stable and can be used to sustain the expression of antigen in cells for longer periods of time than RNA or protein vaccines. Since DNA vaccines cannot amplify by themselves and have often weak immunogenicity, nowadays some strategies are used for enhancing the efficacy of DNA vaccines (reviewed in Fioretti et al (Fioretti et al., 2010).

2. Issues for optimal performance and regulatory compliance of vector design

Vector design issues affect quality, yield and regulatory compliance. Plasmid DNA contains functional elements that afford propagation and selection in a bacterial host organism and elements that drive high level expression in the eukaryotic host (expression cassette comprising eukaryotic enhancer, promoter, terminator/polyadenylation signal) and activate innate immunity. Vector modifications that improve antigen expression (e.g. codon optimization, inclusion of an intron, a strong promoter, exclusion of sequences causing DNA helical instability) are highly correlative with improved immune responses (reviewed in Williams et al. (Williams et al., 2009). These components must be carefully arranged, since a slight modification of a vector to enhance one parameter can have multiple undesired effects on other parameters and ultimately modify immune responses (Ramanathan et al., 2001; Zinckgraf and Silbart, 2003). Improving antigen processing for class I or class II major histocompatibility complex (MHC) presentation can enhance adaptive immune response (reviewed in Leifert et al. (Leifert et al., 2004). Targeting heterologous proteins to various intracellular destinations such as endoplasmic reticulum targeting (Delogu et al., 2000; Gerber et al., 1992; Weiss et al., 1999; Xu et al., 2005) may alter or enhance immune responses.

Immunostimulatory sequences such as unmethylated CpG from bacterial DNA or plasmids can be utilized to enhance T lymphocyte recruitment or expansion via toll like receptor 9 (TLR9) (innate immune responses) and may contribute to the potency of plasmid based DNA vaccines (Liu, 2011).

The overall safety and tolerability of DNA vaccines are substantiated in completed and ongoing clinical trials. Nevertheless, the introduction of plasmid DNA into humans requires special considerations which have been addressed in several recent World Health Organization (WHO), US Food and Drug Administration (FDA), or European Agency for the Evaluation of Medicinal Products (EMEA) regulatory draft guidance's. DNA vaccines that are currently being tested do not show relevant levels of integration into host cellular DNA (Ledwith et al., 2000; Sheets et al., 2006a). Chromosomal integration is considered to be a lower risk with DNA vaccines than conventional vaccines and is not raised as a specific concern in FDA, WHO or EMEA regulatory guidance's (Schalk et al., 2006). Biodistribution and persistence studies are required (EMEA, 2006) and studies examining plasmid biodistribution/persistence/toxicology indicate that DNA vaccines prepared from a common plasmid vector but encoding different antigens behave similarly (FDA, 2007; Schalk et al., 2006). Based on these findings, biodistribution studies may be waived for DNA vaccines produced by inserting a novel gene into a plasmid vector previously documented to have an acceptable biodistribution/integration profile (FDA, 2007), reducing the extent of preclinical testing to validate safety and performance prior to clinical use.

3. DNA vaccine against cancer

The effectiveness to screen for antigens rapidly and to design specific types of expression constructs has made the study of DNA vaccines a valuable field for immunotherapy of cancer.

There is now a range of potential target antigens susceptible to antibody attack, and a multitude of intracellular antigens, requiring cytotoxic T cells.

The DNA vaccine is a prime example of a modern genetic vaccine that not only delivers antigen, but also engages multiple routes to activate innate immunity as well as adaptive immunity against cancer antigens. The mechanisms of action of DNA vaccines and how the encoded antigens can enter the processing and presentation routes of the immune system and activate all effector pathways against cancer are depicted in Fig. 1.

Vaccine design can modify the molecular format of antigen to select the desired effector pathway (Rice et al., 2008). The small amount of antigen produced by DNA vaccines is sufficient for effective priming. The 'prime/boost' strategies, likely to be needed for cancer control, amplify antigen levels (Iurescia et al., 2010b). Electroporation during boosting is an attractive strategy, increasing both antigen levels and inflammatory activity (Iurescia et al., 2010b; Rinaldi et al., 2008).

Moreover, engineering DNA vaccine design for maximizing epitope-specific immunity has allowed the development of the so-called "T cell epitope-driven vaccine design".

4. Epitope-driven vaccine design

The appeal of antitumor vaccination is the fact that it takes advantage of *in vivo* processes and has the potential to harness the full power of the immune system, in contrast to the more artificial *ex vivo* expansion of T cells.

Most immunotherapeutic approaches have centered on the induction of anti-tumor $CD8^+$ T cells, which exhibit cytolityc activity towards tumor cells expressing tumor-specific or tumor-associated Ags. Although cytotoxic T lymphocytes have been deemed to be the key player in the generation of antitumor therapeutic effects, unfortunately immunization strategies focusing only on CTLs remain often suboptimal. $CD4^+$ T cells are critical for the generation and