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Production of epitope-specific antibodies using peptide-CpG-ODN-liposome complex without carriers and their application as a cancer vaccine in mice

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Low efficacy of peptide vaccines limits their potential application. We developed a powerful strategy to produce epitope-specific antibodies using peptides. Immunization with novel formula into mice showed target-specific prophylactic and therapeutic effects against tumors. Our strategy will be useful for rapid eiptope screening, therapeutic antibody production and cancer vaccine development.

Innate immunity and adaptive immunity synergistically protect our bodies from various non-self pathogens such as bacteria, parasites, fungi and viruses. These system also recognize cancer cells as an altered-self and remove them. Adaptive immunity exhibits specificity against antigens through antigen-specific receptors and antibodies. Epitope-based peptide vaccines have been extensively studied in various animal models for the past 30 years and have proved to be pivotal for inducing and regulating immune responses through their binding ability to B-cell receptors and MHC as B-cell epitopes and T-cell epitopes. Peptides are easy to synthesize in a short time and the cost is inexpensive. Therefore, peptide vaccines are potentially useful as prophylaxis for cancers and infectious diseases such as influenza virus, malaria, hepatitis B and HIV.[1] However, the efficacy of peptide vaccines is limited in the treatment of patients. To maximize the magnitude of peptide-driven immune responses, we used a strategy of stimulating the innate immunity with CpG-DNA and facilitated delivery of vaccines using liposomes.[2,3]

CpG-DNA represents synthetic oligodeoxynucleotides (ODNs) and bacterial DNA containing unmethylated CpG dinucleotides flanked by specific base sequences. Because CpG DNA has significant immunomodulatory effects on B lymphocytes, macrophages, dendritic cells and natural killer cells, it has gained attention for its potential use as an immune adjuvant. Many investigators have utilized phosphorothioate-modified types of CpG-DNA (PS-ODN), which are resistant to nuclease activity and can be efficiently delivered into cells.[4] However, PS-ODN induces backbone-related side effects, such as transient splenomegaly, arthritis and PS-ODN-specific IgM production in PS-ODN-treated mice.[5] Therefore, investigators, including us, have developed phosphodiester bond CpG-DNA (PO-ODN) as a natural counterpart of PS-ODN to induce optimal innate immune responses. We screened natural PO-ODN with immunomodulatory activity from *Mycobacterium bovis* genomic DNA and isolated a potent PO-ODN, namely MB-ODN 4531(O), which contains three CpG motifs and functions as a powerful adjuvant without causing severe side effects in mice.[6] In contrast to PS-ODN, PO-ODN shows an immunomodulatory effect only in mouse cells and not in human cells.[7] Liposomes are potent vehicles for delivering antigens to antigen presenting cells and are known to enhance antibody production and cytotoxic T lymphocyte (CTL) responses.[8] To enhance the potency of PO-ODN in human cells, we tested several different formulas of liposome complexes and found a special composition of choice: phosphatidyl-β-oleoyl-γ-palmitoyl ethanolamine (DOPE)/cholesterol hemisuccinate (CHEMS) (1:1 ratio). The natural CpG-DNA, in combination with the DOPE/CHEMS complex (Lipoplex(O)), increased expression of cytokines such as IL-6, IL-12 and IFNγ in human cells, as well as in mouse cells, through improved intracellular uptake of CpG-DNA and TLR9/MyD88-mediated cellular activation. Levels of cytokine expression in mouse serum were also elevated by Lipolex(O) in TLR9-dependent and CpG-sequence dependent manners. Furthermore, Lipoplex(O) had an adjuvant activity when proteins such as hen egg lysozyme and ovalbumin were used as antigens in mice. Therefore, we next

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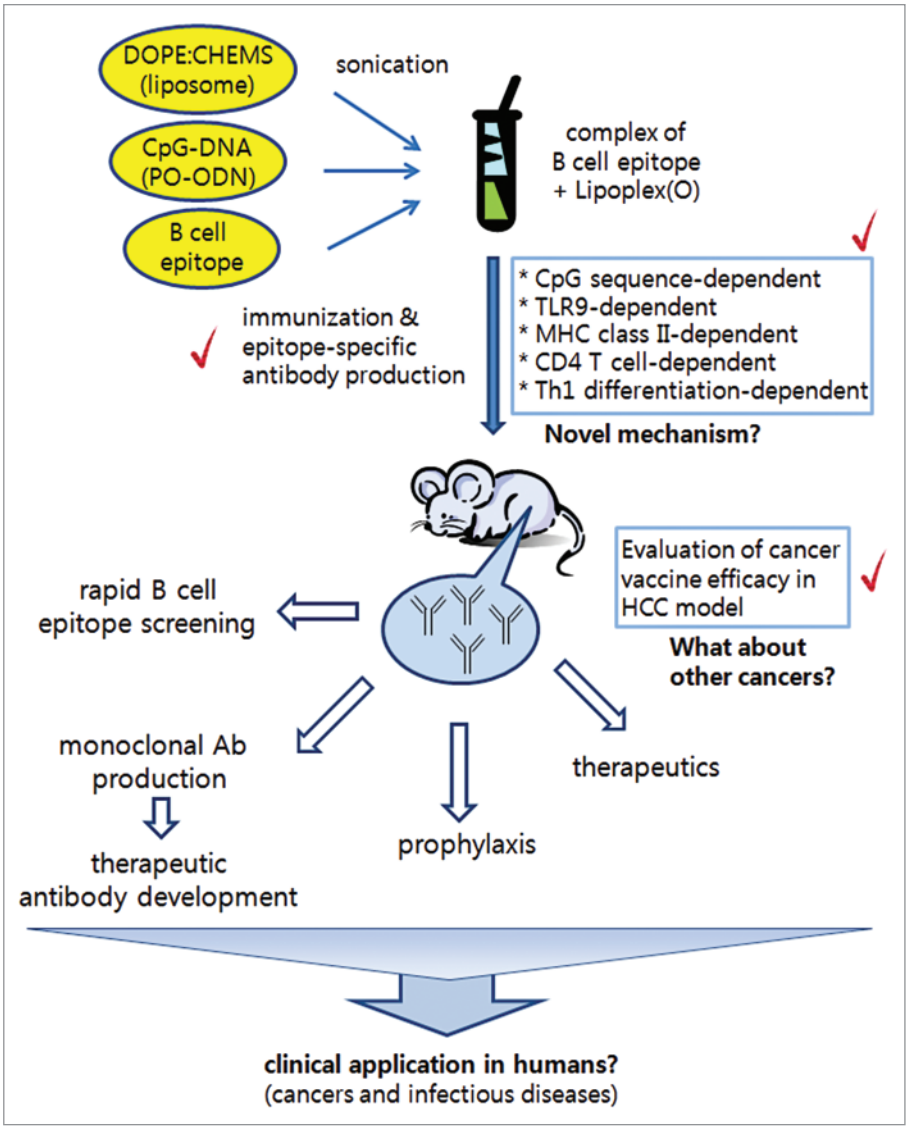

Figure 1. Composition of novel peptide vaccines, some key mechanisms, evaluation in mice, and their possible application. Our peptide vaccine is a complex consisting of B cell epitope peptide, natural phosphodiester CpG-DNA and DOPE:CHEMS liposome mixture. Immunization with the peptide vaccine in mice induces production of epitope-specific antibodies. Based on our experimental results, this process involves CpG-DNA, TLR9, MHC-II, CD4 T cells and Th1 differentiation. Further investigation of the functional mechanism will give us a better understanding of the powerful efficacy of our novel peptide vaccine. We confirmed prophylactic and therapeutic effects with peptide vaccine targeting a cancer-specific antigen in the mouse hepatocellular cancer (HCC) model. We believe that this strategy can be widely used in rapid epitope screening, therapeutic antibody development, cancer vaccines and defense against infectious diseases.

tried to apply Lipoplex(O) for a synthetic peptide-based B cell epitope screening and antibody production using peptides as an antigen.[2]

We predicted candidate B cell epitopes from a cancer specific antigen and viral antigens using computer algorithms and injected each epitope along with Lipoplex(O), into mice; we then checked for the production of epitope-specific antibodies.[2,3,9] The results revealed that our strategy is very useful for the selection of potent B cell epitopes. To further evaluate our strategy in detail, we focused on studying the use of a B cell epitope peptide (TM4SF5R2–3) that we screened from hepatocellular carcinoma (HCC)-specific transmembrane 4 superfamily member 5 (TM4SF5) protein.[10] Complexes of peptide and Lipoplex(O) significantly enhanced peptide-specific IgG production depending on TLR9, $CD4^+$ T cell, MHC-II and Th1 cell differentiation. Considering that we are using only B-cell epitope without any carrier, the involvement of the $CD4^+$ T cell and MHC-II is a very interesting characteristic of the response. Even though we do not know the exact mechanism involved in this process, our powerful strategy to produce epitope-specific antibodies without the need of a carrier protein has a clear advantage (**Fig. 1**). Monoclonal antibody produced by immunization with a complex consisting of TM4SF5R2–3 and Lipoplex(O) inhibited the growth of HCC cells expressing the antigen. Furthermore, immunization with a complex consisting of TM4SF5R2–3 and Lipoplex(O) protected mice from mouse HCC cell implantation in a TLR9-dependent manner. Preimmunization with our vaccine induced robust production of peptide-specific antibodies after cancer cell challenge and significantly suppressed tumor growth (tumor weight 2.5 g vs. 0.25 g), proving its prophylactic effect. Immunization after cancer cell implantation also significantly suppressed tumor growth (0.8 g vs. 0.25 g) and increased survival rate (0% vs. 100% at 100 d after cancer cell implantation) revealing the method's therapeutic effect. Therefore, we conclude that our peptide vaccine works successfully without apparent side effects in the mouse cancer model.[3] Because functional antibodies produced using this strategy can be applied as therapeutics for cancers after the humanization process, the experiment is ongoing. Further investigation of the functional mechanism of our novel vaccine will shed more light on the rationale of our vaccine.

We believe that our strategy will contribute to a more effective defense against life-threatening diseases. Recently, the appearance of novel viruses and the globalization of the world have created extended threats to human beings. Our novel strategy can be promptly used in rapid screening of potent B-cell epitopes and can enable timely defenses against pandemics of infectious diseases. It can potentially be applied for the development of therapeutic antibodies against exposure to bioterrorism agents. Evaluation of our vaccine in cancers other than HCC will further expand applications.

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