Human Vaccines

ISSN: 1554-8600 (Print) 1554-8619 (Online) Journal homepage: informahealthcare.com/journals/khvi19

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To cite this article: Ching-Liang Chu, Dz-Chi Chen & Chi-Chen Lin (2011) A novel adjuvant Ling Zhi-8 for cancer DNA vaccines, Human Vaccines, 7:11, 1161-1164, DOI: 10.4161/hv.7.11.17753

To link to this article: https://doi.org/10.4161/hv.7.11.17753

Published online: 01 Nov 2011.

Human Vaccines 7:11, 1161-1164; November 2011; © 2011 Landes Bioscience

A novel adjuvant Ling Zhi-8 for cancer DNA vaccines

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DNA vaccines have a wide range of applications, with several potential advantages compared with other vaccine technologies for diseases. No DNA vaccine has yet been licensed in humans; however, a lot of effort has been made to enhance their potential as human vaccines and therapeutics. Finding an effective adjuvant is a strategy to improve the efficacy of DNA vaccines. We recently identified a fungal immunomodulatory protein Ling Zhi-8 (LZ-8) with stimulatory activity on dendritic cells (DCs) that significantly increases the efficacy of a cancer DNA vaccine in a preclinical tumor model, suggesting that LZ-8 may be a good candidate adjuvant for vaccine development. Here we discuss the possibility for applying LZ-8 to a cancer DNA vaccine for humans.

Key words: cancer, DNA vaccine, adjuvant, LZ-8, fungal immunomodulatory protein, dendritic cell

Submitted: 08/08/11

Accepted: 08/14/11

DOI: 10.4161/hv.7.11.17753

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DNA Vaccines for Cancer Therapy

History of DNA vaccine development. Traditionally vaccines were prepared by the inactivation of infectious pathogens or the isolation of antigenic proteins. Following the initiation of recombinant DNA technology, the production of antigens became relatively easy, and the concept of DNA immunization was promoted. In 1992, Tang et al. first showed that an immune response could be induced by introducing a plasmid DNA containing the gene encoding an antigenic protein into mouse skin.[1] Then immunization with a plasmid DNA expressing influenza virus hemagglutinin glycoprotein was demonstrated to provide protection against a lethal influenza challenge.[2]

Compared with traditional protein vaccines, DNA vaccines offer several advantages, including easy preparation and storage stability. A successful example is a DNA vaccine against the West Nile virus, which was developed in one month.[3] Thus, the rapid preparation of a DNA vaccine may provide for a rapid-response therapy. According to the success of a DNA vaccine for an infectious disease in animals, the idea for its use in the prevention and treatment of cancer was stimulated.[4]

Development of DNA vaccines as a new strategy for cancer therapy. To use a DNA vaccine for cancer therapy, the antigen encoded by the DNA vaccine is the critical factor for inducing specific immune responses. The targeting antigens in cancer can be divided into exogenous and endogenous antigens. Exogenous antigens are found in virus-associated cancer, such as cervical cancer and hepatocellular carcinoma. For example, human papilloma virus (HPV) is critical for the development of cervical cancer and immunization with a DNA vaccine may potentially eradicate benign lesions and malignant tumors by generating immunity against early viral proteins. In addition, therapeutic HPV DNA vaccines have been shown to induce anti-tumor immune responses in preclinical animal models. Several DNA vaccines are in clinical trials for testing therapeutic efficacy.[5]

In contrast to the strong immune responses generated against exogenous tumor antigens, the endogenous antigens, i.e., tumor-associated self-antigens, are usually weakly immunogenic. Although passive immunotherapy has

been documented to be clinically effective, as has been demonstrated by the use of anti-HER2/neu antibody in neu-overexpressed breast cancer,[6] there have been only limited examples of effective induced immunity against cancer. However, cellular or humoral immune responses against certain tumor-associated antigens can be detected in human patients, which suggests that the low reactivity of the particular immune cells toward endogenous antigens may be boosted by an immunological stimulus.[7]

The presence of immune cells (type, density and location) within tumor samples was found to be a better predictor for patient survival than histopathological methods.[8] Therefore, induction of cellular immunity against cancer may be a potential strategy in cancer therapy. In this aspect, DNA vaccines, compared with protein-based vaccines, provide a rational choice to generate antitumor responses, since DNA vaccines can induce both humoral and cellular immunity.[9]

Manipulation of DNA vaccine. For construction of a DNA vaccine, an antigen-encoded gene is usually placed under the control of a eukaryotic promoter. The cytomegalovirus immediate-early promoter is mostly used due to its high transcriptional efficiency in many cell types. In addition to the essential expression elements, plasmid DNA contains several unmethylated CpG motifs that can interact with Toll-like receptors and regulate immune responses. An excellent comprehensive review on the development of DNA vaccines is given by Signori et al.

Current problem of DNA vaccine. Although DNA vaccines are effective in inducing immune responses in small animal models, the immunogenicity of DNA vaccines in non-human primates and humans is slow.[11] Therefore, it is a major challenge to develop novel approaches to circumvent this problem in the development of cancer DNA vaccines. Several strategies have been proposed and tested;[12] however, we are interested in identifying a novel adjuvant for enhancing the immunogenicity of a cancer DNA vaccine.

Adjuvants for Cancer DNA Vaccines

Adjuvants in vaccine development. An immunologic adjuvant is an agent that can stimulate the immune system and increase the immune response to a vaccine antigen. The study of adjuvants has become important to the development of new subunit vaccines consisting of purified or recombinant antigens.[13] Alum is the most successful and widely used adjuvant in human vaccines and has been particularly effective for promoting humoral immune responses. However, alum has little effectiveness in inducing cell-mediated immune responses, especially cytotoxic T-cell responses required for protection against cancer.[14] Thus, the goal of understanding immunology and particularly the innate immune system is guiding the development of novel and specifically directed adjuvant strategies. A recent review by Schijns et al. addresses the importance of adjuvants in vaccine development.[15]

Adjuvants used in cancer DNA vaccines. A number of adjuvants have been employed in cancer DNA vaccines, including chemokines, activating cytokines, costimulatory molecules, DC-targeting antibodies and molecules to manipulate antigen presentation and/or processing.[12] For example, 1P7-Ig, an Ig-fused CCR5 superagonist derived from natural CCL5, is used as an adjuvant for cancer DNA vaccines and has been shown to induce a strong Th1 responses against tumor growth.[16] Despite the success of currently tested adjuvants for generating immune responses to cancers, improving adjuvanticity or searching for novel adjuvants remains an important issue for enhancing the efficacy of cancer DNA vaccines..

LZ-8: A Potential Adjuvant from Nature Products

Discovery of LZ-8. The medicinal properties of *Ganoderma lucidum* (*G. lucidum*; Ling Zhi or Reishi), a well-known lamella-less basidiomycetous fungus, have been recognized for many centuries in Asia.[17] A number of pharmaceutically active compounds have been isolated from *G. lucidum*.[18] In addition to polysaccharides and triterpenes/triterpenoids compounds, fungal immunomodulatory protein (FIP) is an important bioactive component with immune regulating activity in mushroom.[19] Thus, Ling Zhi-8 (LZ-8) was identified from *G. lucidum* mycelia,[20] and then was sequenced and cloned.[21]

Immunomodulatory effect of LZ-8. After the discovery of LZ-8, researchers began to reveal its bioactivities. Some studies have shown the immunomodulatory effect of LZ-8 on autoimmunity and transplantation;[22] LZ-8 facilitates cellular interaction through modulation of adhesion molecules.[23] In addition, LZ-8 can work as a mitogen to activate T cells.[24] Jeurink et al. reported that protein extracts of *G. lucidum* contain immunomodulating activity by acting directly on monocytes,[25] implying that proteins including LZ-8 in *G. lucidum* may stimulate myeloid cells. However, many other immunoregulatory functions of LZ-8 remain to be explored.

Stimulatory activity of LZ-8 on dendritic cells (DCs). Recently, LZ-8 was reported to activate human and mouse DCs.[26,27] DCs are professional antigen-presenting cells and work as a bridge to link innate and adaptive immunity.[28] When stimulated by inflammatory mediators or microbial pathogens, DCs migrate to the peripheral lymphoid organs and become mature, which dramatically enhances the ability of DCs to activate antigen-specific T cells.[29] Because of their key regulatory role in immune responses, DCs are being developed as potent new vaccines for the treatment of cancer and viral infections.[30] Thus, LZ-8 can potentially be applied to immunotherapy and vaccination.

Is it Possible to Apply LZ-8 with a DNA Vaccine in Humans?

We demonstrated very recently that LZ-8 enhances the efficacy of a cancer DNA vaccine via activating DCs.[27] Our report provides a new target for adjuvant development and implies the potential for

LZ-8 and DNA vaccine in cancer therapy. However, much effort is needed before applying this approach to humans.

Clinical trials of DNA vaccine for cancer therapy. Before being licensed for humans, candidate vaccines have to be tested for safety, tolerability and efficacy in various clinical trials. Many cancer DNA vaccines have been evaluated in various phases of clinical trials.[12] All results have demonstrated that DNA vaccines are well tolerated and safe. Since our preclinical model is using HER-2/neu DNA vaccine against p185(neu) expressing tumor MBT-2 in mice, we are especially excited by phases I and II clinical trial data showing that patients with HER2/neu overexpressing breast and ovarian cancers have detectable antibody and T-cell immunity against HER2/neu after vaccination.[31] Thus, the use of our cancer DNA vaccine in humans should be possible soon.

Current status of LZ-8 development. Recombinant LZ-8 (rLZ-8) has been generated in many expression systems, but the large-scale amplification of rLZ-8 has been achieved in a patented yeast system. This has been a good source for LZ-8, and the cGMP process is being developed. The major advantage of LZ-8 as an adjuvant candidate is to significantly promote specific Th1 responses, which is required for cancer therapy. A polysaccharide extract of *G. lucidum* also has been reported as a potential adjuvant.[32] However, LZ-8 can be easily produced and quality controlled when compared with the extract. Another protein adjuvant, heat shock protein70 (HSP70), has been tested in recent clinical trials of a HPV DNA vaccine.[33] This work has provided a valuable clue for the application of LZ-8 in humans. It will be interesting to compare LZ-8 to HSP70, including immunological effects, routes of application and formula.

In conclusion, we have provided evidence for applying LZ-8 with a cancer DNA vaccine for cancer therapy. Although our cancer DNA vaccine should be soon in humans, the use of LZ-8 in the clinic will take additional time. However, we believe that the application of LZ-8 could be hastened if an experienced vaccine or pharmaceutical company is interesting in developing LZ-8 as an adjuvant, not only for use in DNA vaccines but also in other types of vaccine.

Conflict of Interest

A patent for the use of LZ-8 as an adjuvant in vaccination has been filed.

Acknowledgements

We would like to thank all members in Drs. Ching-Liang Chu and Chi-Chen Lin laboratories for their technical help. We also thank Yeastern Biotech Co. Ltd., for providing LZ-8.

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