Therapeutic Vaccines for Hypertension and Dyslipidemia

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SUMMARY

Vaccines are commonly used as a preventive medicine for infectious diseases worldwide, however, clinical trials on an amyloid beta vaccine for Alzheimer’s disease represents a new concept in the field of vaccinations. Several recent studies indicate the potential of therapeutic vaccines as well as classical vaccines as preventive medicines. A number of therapeutic vaccines for cancer have been developed as novel immunotherapies. Their targets are usually specific antigens in cancer cells, allowing activated cytotoxic T cells (CTLs) to attach and remove the antigen-presenting cancer cells. Recently, we and others have attempted to develop a therapeutic vaccine against hypertension. The vaccine target is angiotensin II (AngII), and induced anti-AngII antibodies could efficiently ameliorate high blood pressure. However, because AngII is an endogenous hormone, we must avoid the induction of autoimmune diseases by administration of an AngII vaccine. Therefore, our system was used to design a therapeutic vaccine that elicits anti-AngII antibodies without CTL activation against AngII. Because the target antigen itself does not include T cell epitopes, the immunogenic molecule (ie, KLH) provides antigen that supports the activation of T cells. In particular, helper T cells may activate B cells that produce antibodies against our specific antigen. In this review, we will explain our concept of therapeutic vaccines based on our recent data. (Int Heart J 2014; 55: 96-100)

Key words: Angiotensin II, Lipoprotein(a)

Vaccines are commonly used worldwide to protect against infectious diseases, and clinical trials for amyloid beta vaccines against Alzheimer’s disease, cancer and rheumatoid arthritis will usher in a new era in vaccination. We will pursue the development of a vaccine for patients with high blood pressure. The number of patients with hypertension increases each year, and several types of antihypertensive drugs are available for treatment. Antihypertensive drugs are effective with few side effects, however, patients often need to take two or three drugs at a time to control severe hypertension. Therefore, the increased medical costs associated with treating hypertension might become a fiscal and social problem that affects social security expenses. The ultimate goal of our study is to reduce medical costs through the clinical use of a therapeutic vaccine for hypertension. However, when considering clinical applications, the safety of therapeutic vaccines should be carefully examined. In the history of therapeutic vaccines, the amyloid beta vaccine effectively reduced amyloid plaques and restored memory function in several animal models of Alzheimer’s disease. Unfortunately, however, the clinical trial for this vaccine was halted because 6% of the participants developed aseptic meningoencephalitis, even though amyloid plaque reduction was observed. Postmortem examination of the brains of two patients who suffered from aseptic meningoencephalitis due to the vaccine revealed T lymphocyte infiltration into the brain.

Based on this previous experience, we must develop a plan to balance safety and efficacy. In this review, we will describe the concept of our therapeutic vaccination in the first part and introduce the history of hypertension and dyslipidemia vaccines in the second part.

Concept of our therapeutic vaccine: Most antihypertensive drugs are safe and effective for patients. Therefore any vaccine for treating hypertension must also be highly safe and effective. In the case of infectious diseases and cancer, the targets of vaccines are bacteria, viruses and cancer cells that must be killed by an immune reaction. However, the antigens for a hypertension vaccine are usually endogenous hormones (ie, angiotensin II) that are not foreign entities. Therefore, we have to design a vaccine system that avoids inducing autoimmune disease.

Our immune system can distinguish self from non-self because the immune system has evolved central and peripheral self-tolerance checkpoints to remove or silence autoreactive lymphocytes. B cells that react to self-antigens become anergic and functionally silent. In order for dormant B cells to proliferate, B cells need to interact with activated T-helper cells recognizing antigen-derived peptides on major histocompatibility complex (MHC) class II molecules presented by B cells. T cells that react with self-antigen, having escaped thymic deletion and existing in peripheral lymphoid organs, may be activated by antigen stimulation in the presence of strong adju-
AngII has been used as an AngII peptide vaccine in mice. The results indicated that AngII-KLH and KLH induced T cell activation but AngII did not, which means that KLH contains a T-cell epitope, but AngII does not. Importantly, the sources of T-cell epitopes and B-cell epitopes can be different. This situation is reflected in the relationship between a hapten and its carrier, in which the hapten has only B-cell epitope and the carrier possesses the T-cell epitope. Based on this mechanism for our therapeutic vaccine system, autoimmune diseases caused by cytotoxic T cells can be avoided.

**Therapeutic vaccine for hypertension:** Vaccines for hypertension, targeting the renin-angiotensin system, have been reported since the 1950s. A renin vaccine was reported to successfully reduce blood pressure by some groups. However, since Michel, *et al.* reported that the vaccine induced autoimmune disease of the kidneys in two animal models, no further research on a renin vaccine has been reported. An angiotensin I vaccine (PMD3117) reduced blood pressure in rat and mouse models. However, the vaccine did not reduce blood pressure in a clinical trial. The reason for the failure was considered to be the feedback pathway between angiotensin II (AngII) and renin. An AngII vaccine (AngQb-Cyt006) was reported to be effective at producing anti-AngII antibodies in both rodents and humans. AngQb-Cyt006 is a conjugate vaccine, composed of angiotensin II chemically linked to recombinant virus-like particles derived from the RNA-phage Qφ. In clinical trials, AngQb-Cyt006 was injected at 0, 4, and 12-week dosing intervals. In this multicentre, double-blind, randomised, placebo-controlled phase IIa trial, 72 patients with mild-to-moderate hypertension were randomly assigned with a computer-generated randomisation list to receive subcutaneous injections of either 100 μg CYT006-AngQb (n = 24), 300 μg CYT006-AngQb (24), or placebo (24), at weeks 0, 4, and 12. Twenty-four hour ambulatory blood pressure was measured before treatment and at week 14. In the 300 μg group, there was a reduction from baseline in mean ambulatory daytime blood pressure at week 14 by –9/–4 mmHg compared with placebo. The 300 μg dose reduced the early morning blood-pressure surge compared with placebo (~25–~13 mmHg). However, further clinical studies of the AngII vaccine failed to reproduce the blood pressure reduction, despite shorter dosing intervals (0, 2, 4, 6 and 10 weeks) and higher antibody titers. Therefore, the development of an AngII vaccine for clinical application has now been halted.

We have also examined the potential of an AngII vaccine in mice. The anti-AngII antibody production of B cells was evaluated in response to vaccination with AngII, KLH and AngII-KLH with or without adjuvant. As a result, only AngII-KLH with adjuvant successfully induced the production of anti-AngII antibodies. The antibody titers in the AngII-KLH with adjuvant group increased as the dose of the antigen increased, and the anti-AngII antibody recognized AngII to a lesser extent, although it did not recognize angiotensinogen. These results suggest that vaccination with AngII-KLH and adjuvant can produce antibodies specific for AngII in C57Bl/6 mice. We further examined the time course of anti-AngII antibody titers at 42, 70, and 98 days after vaccination with AngII-KLH. The antibody titer peaked on day 42 and decreased on days 70 and 98 in the same manner as previously reported. To examine whether B cells could be activated by AngII itself, we compared the antibody titers in mice prior to AngII infusion (day 42) with titers obtained after AngII infusion (day 56). The post-
infusion titer was lower than the pre-infusion titer. In addition, the post-infusion titer decreased in the same manner as in the titer with no AngII infusion. This result suggests that endogenous AngII does not stimulate antibody production, even after AngII has been recognized as a target by the immune system.

To evaluate the effect of immunization in mice, we examined the effect of high-dose (1,000 ng) or low-dose (100 ng) AngII-KLH vaccination in mice using an AngII infusion model. Interestingly, at steady state, mice immunized with either a high dose or a low dose of AngII-KLH failed to show lower blood pressure than control mice. After AngII infusion (1,000 ng/kg/minute), the systolic blood pressure was increased in the control mice and, to a lesser extent, in the low dose-immunized mice but not in the high dose-immunized mice. Thus, mice immunized with a high dose of AngII-KLH exhibited a significant decrease in systolic blood pressure compared to the control mice. We further analyzed the correlation between anti-AngII antibody titers and systolic blood pressure. A negative correlation was observed between the anti-AngII antibody titer and systolic blood pressure in the high-dose and low-dose immunized mice after AngII infusion. This result indicates that the antibody elicited by the vaccine efficiently reduces blood pressure in an AngII infusion model. Furthermore, we examined the degree of cardiac hypertrophy and fibrosis after AngII infusion in high-dose immunized mice and control mice. Systemic AngII treatment caused myocardial hypertrophy in control mice, but immunized mice exhibited an amelioration of the AngII-induced increase in the heart weight to body weight ratio. Similarly, AngII treatment led to the development of perivascular fibrosis in the hearts of control mice, but immunized mice exhibited less fibrotic changes. These results revealed that the anti-AngII antibody induced by AngII-KLH vaccination efficiently attenuated AngII-induced signaling in vitro and AngII-induced hypertension and cardiac remodeling in vivo.\(^{12}\)

**Therapeutic vaccine for other lifestyle-related diseases:** Similar to hypertension, dyslipidemia is also a candidate target for a therapeutic vaccine. As is well-known, atherosclerosis is produced by an inflammatory response, which is triggered by the accumulation of lipoproteins over the arterial vessels. The current therapeutic strategy consists of decreasing LDL (low-density lipoprotein) levels with a consequent increase in HDL (high-density lipoprotein) levels, which is performed by pharmaceutical medications (ie, statins). The vaccination approach has been attempted by targeting a few endogenous proteins related to atherosclerosis such as Apolipoprotein B100 (ApoB100), the main component of LDL, and cholesterol ester transferase protein (CETP), which converts cholesterol esters and triglycerides from HDL to LDL.\(^{23-30}\) While studying the ApoB100 vaccine, Nilsson’s group determined which epitopes are the products of LDL oxidation and reduced atherosclerosis in apoE (apolipoprotein E) knockout mice using p45 epitope vaccine.\(^{31}\) This idea is excellent with regards to creating a novel immunotherapy for atherosclerosis; however, the humoral and cellular immune responses to such clinical applications have not been fully elucidated. The CETP vaccine is also a promising approach with which to increase HDL cholesterol. Rittershaus’s group designed a chimeric peptide vaccine that contains a T-cell epitope of tetanus toxin and a B-cell epitope from part of human CETP (TT-CETP vaccine).\(^{29}\) This vaccine treatment for hyperlipidemia significantly decreased LDL and increased HDL in rabbits. Based on these animal experiments, phase I and II clinical trials have been conducted.\(^{32}\) As a result, half of the patients who received TT-CETP vaccine developed anti-CETP antibody; however, the titer levels of anti-CETP antibody were not sufficient to increase HDL. In the clinical study, this therapeutic vaccine was well tolerated and no significant abnormalities were observed, which might suggest the safety of this therapeutic vaccine in phase I trials. Although a CETP inhibitor has been considered a promising drug, it failed the phase II clinical trials due to a lack of efficacy. Therefore, the therapeutic target of this vaccine has been reconsidered.

We administered the apo(a) vaccine to patients with high lipoprotein(a) [Lp(a)].\(^{33}\) Lp(a) is a unique plasma lipoprotein that consists of a cholesterol-rich LDL particle with one molecule each of apolipoprotein B-100 (apoB) and apolipoprotein (a) [apo(a)], which are bound through a single disulfide bond.\(^{34}\) Lp(a) is found only in humans, primates and hedgehogs. Apo(a) is a homolog of plasminogen\(^{35}\) that contains 10 different types of plasminogen kringle-4-like repeats (kringle-4 types 1 through 10) and regions homologous to the kringle-5 and inactive protease regions.\(^{36}\) Lp(a) is considered to be an independent cardiovascular risk factor because numerous studies have demonstrated the potent positive association between plasma Lp(a) levels and cardiovascular disease/coronary artery disease. Increased Lp(a) levels are believed to promote atherosclerosis via Lp(a)-derived cholesterol entrapment in the intima, inflammatory cell recruitment, and/or the binding of pro-inflammatory oxidized phospholipids.\(^{37}\) Lipid-lowering agents such as statins have little or no effect on plasma Lp(a) levels.\(^{35}\) Although niacin or estrogen might reduce plasma Lp(a) levels slightly, there is no specific agent to reduce plasma Lp(a) or prevent Lp(a)-induced atherosclerosis. Thus, to inhibit the biological activity of Lp(a), we developed a DNA vaccine for apo(a) that targets 12 selected hydrophilic amino acids in the kringle-4 type 2 domain of apo(a) (Figure 2).\(^{38,39}\) Hepatitis B virus core protein was used as an epitope carrier to enhance the immunogenicity. Intramuscular immunization with apo(a) vaccine resulted in significant inhibition of neointima formation in a carotid artery ligation model using Lp(a) transgenic mice,
which was associated with anti-apo(a) antibody and a decrease in vascular Lp(a) deposition. In this study, we selected 12 amino acids of the kringle-4 type 2 repeat domain of apo(a) that did not share sequence homology with plasminogen. This 12-amino acid motif was incorporated into the hydrophilic domain of the B-cell epitope, which has been used previously to induce an immune response. We expected that the antibody that was produced by this construct could potentially bind not only to free apo(a) in the plasma but also to apo(a) associated with apoB in LDL. If free apo(a) were captured by the anti-apo(a) antibody, vaccination against apo(a) might inhibit atherosclerosis by reducing the deposition of Lp(a) in the atherosclerotic artery or neutralizing apo(a) or Lp(a). Interestingly, apo(a) DNA vaccination markedly decreased the neointima formation induced by artery ligation, which was associated with a decrease in the vascular accumulation of Lp(a) in Lp(a) transgenic mice. Unexpectedly, serum Lp(a) levels were not decreased in this study even though the vaccine against apo(a) successfully produced neutralizing antibodies against Lp(a). Similar findings have been previously reported for another vaccine: Ang II vaccination did not decrease AngII levels in mice. Alternatively, it is possible that the ELISA used in this study could not distinguish between free Lp(a) and antibody-bound Lp(a) because the detecting antibody targets a different epitope than the induced antibody. Further study will be necessary to answer this question.

We have begun to investigate the development of therapeutic vaccines for lifestyle-related diseases. Toward clinical application, the design of clinical trials will be important to prove our proof of concept in the clinical setting. To achieve the reduction of medical costs by the clinical use of a therapeutic vaccine, we will address common diseases as well as severe diseases as a target of therapeutic vaccines in the future. We hope that this novel concept of this study will contribute to promoting health and medicine in the future.

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DISCLOSURE

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