Original Article

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A novel angiotensin II peptide vaccine without an adjuvant in mice

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Objectives: We recently developed a novel peptide, AJP001, that possesses both a mouse T-cell epitope and adjuvant action. Direct conjugation to the antigen is useful for peptide vaccines without the addition of adjuvants. In this study, the efficacy of an angiotensin (Ang) II and AJP001-conjugated peptide vaccine (AJ-Ang II) was evaluated in mice.

Methods: The anti-Ang II antibody titer was measured in Balb/C mice following three injections of AJ-Ang II at 2-week intervals. SBP was measured during vaccination of Balb/C mice treated with Ang II infusion (1 μg/kg per min).

Results: AJ-Ang II treatment resulted in an increase in the anti-Ang II antibody titer in a dose-dependent manner without the addition of adjuvants. In the analysis of the humoral immune response, AJ-Ang II mainly elicited IgG1 antibodies and IL-4 and IL-10 production, as measured by an enzyme-linked immune absorbent spot assay, which suggests the induction of a Th2 response. Importantly, cotreatment with purified antibodies attenuated Ang II-induced extracellular signal-regulated kinase phosphorylation and nuclear factor (NF)-κB activation in cultured vascular smooth muscle cells. The SBP in immunized mice was significantly lower than that in nonimmunized mice (135.9 ± 8.5 vs. 154.9 ± 16.8 mmHg, $P = 0.02$). Furthermore, Ang II-induced perivascular fibrosis in the heart was significantly attenuated in immunized mice, which also exhibited decreased mRNA expression of collagen I/III and transforming growth factor-β.

Conclusion: AJ-Ang II may be a simple and useful therapeutic peptide vaccine without the addition of any adjuvants.

Keywords: AJP001, angiotensin II, hypertension, T-cell epitope, vaccine

Abbreviations: AJ-Ang II, Ang II and AJP001-conjugated vaccine; Ang, angiotensin; AT1R, Ang II type 1 receptor; BP, blood pressure; ELISPOT, enzyme-linked immune absorbent spot; ERK, extracellular signal-regulated kinase; TGF-β, transforming growth factor-β; VSMC, vascular smooth muscle cell

INTRODUCTION

Hypertension is a major cause of cardiovascular diseases, including strokes, heart diseases and kidney diseases. It is widely known that blockers of the renin–angiotensin (Ang)–aldosterone system, including Ang-converting enzyme inhibitors and Ang II type 1 receptor (AT1R) antagonists, are cornerstones for the treatment of hypertension. Although we have many therapeutic options, a substantial portion of the hypertensive population has uncontrolled blood pressure (BP). In particular, medication nonadherence is one of the major causes of uncontrolled BP [1–3]. Therefore, a treatment option that is independent of adherence would be ideal.

We have demonstrated the efficacy of an Ang II vaccine in reducing high BP in mice and spontaneously hypertensive rats [4,5]. In general, vaccines that induce antibodies against self-antigens require carrier proteins, such as keyhole limpet hemocyanin; a virus-like particle; and an adjuvant because self-antigens themselves are poorly immunogenic [6]. As carrier proteins are highly immunogenic, they are effective in the induction of antibodies against coupled B-cell epitopes. However, carrier proteins have several problems: difficulties in controlling the uniformity of the coupling process and provoking undesirable immune responses, such as allergy and anaphylaxis. From this background, we recently developed a novel peptide, AJP001 (Ac-ELKLIFLHRLKRLRKRLKRK-amide), which behaves as a T-cell epitope in mice and humans, functioning as a peptide vaccine without the addition of adjuvants [7]. Herein, we investigated the efficacy and safety of an Ang II and AJP001-conjugated vaccine (AJ-Ang II) in mice.

METHODS

Vaccine synthesis and preliminary experiments

Experiments were approved by the Ethical Committee for Animal Experiments of the Osaka University Graduate School of Medicine. The AJ-Ang II and an Ang II and BSA conjugate (BSA-Ang II) were synthesized by the

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Peptide Institute, Inc. (Osaka, Japan). Six-week-old male Balb/C mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). All mice were housed in a temperature-controlled and light cycle-controlled facility. In preliminary experiments to determine the appropriate vaccine dose, mice were divided into a high-dose vaccine group (1000 μg/mouse AJ-Ang II, $n=4$), a low-dose vaccine group (100 μg/mouse AJ-Ang II, $n=4$) and a control group (1000 μg/mouse AJP001, $n=4$). Mice were immunized subcutaneously three times at 2-week intervals. Blood samples were collected during weeks 0, 2, 4, 6, 8 and 10. Serum samples were isolated and stored at −70 °C until use.

Angiotensin II infusion and blood pressure measurement

Following the preliminary experiments, mice were divided into a vaccine group (1000 μg/mouse AJ-Ang II, $n=8$), a control group (1000 μg/mouse AJP001, $n=8$) and a saline group ($n=8$). Ang II (1 μg/kg per min) was continuously infused through osmotic mini-pumps (Muromachi Kikai Corporation, Tokyo, Japan) at 6 weeks. The arterial BP was measured at 4, 7 and 10 weeks by the tail-cuff method (BP-98A, SOFTRON, Tokyo, Japan). SBP values are shown as the average of 10 readings for each animal at each time point. The mice were euthanized during week 10 (Fig. 1a).

ELISA

Ang II-specific antibody responses were measured with ELISA plates coated with BSA-Ang II at 10 μg/ml in carbonate buffer overnight at 4 °C. After blocking with a 5% skim milk solution in PBS/Tween, serum samples from immunized animals were diluted 1 : 100 in PBS containing 5% skim milk and then incubated for 2 h on the plate. Detection was performed with an antimouse IgG horseradish peroxidase conjugate (NA935; GE Healthcare, Chicago, Illinois, USA). The optical density (OD) (450 nm) was read on a microplate reader (Bio-Rad Inc., Hercules, California, USA). The antibody titer is expressed as the serum dilution that exhibited half-maximal binding. Furthermore, antimouse IgG subclass-specific horseradish peroxidase-conjugated antibodies (IgG1 and IgG2a) were used for the IgG subclass determination assay.

Enzyme-linked immune absorbent spot assay

The details of the enzyme-linked immune absorbent spot (ELISPOT) assay were described previously [7]. Splenocytes were isolated from mice in the vaccine group and saline group, added to plates (5×10^5 cells per well) and stimulated with 10 μg/ml Ang II or AJ-Ang II at 37 °C for 48 h. Phorbol myristate acetate (PMA) and ionomycin were added as positive controls at a concentration of 100 ng/ml each. Spots were counted using a stereomicroscope (Stemi 305; Carl Zeiss Microscopy Co., Ltd., Jena, Germany).

Plasma angiotensin II concentration measurement

The plasma Ang II concentration was measured using an Ang II ELISA kit (Enzo Life Sciences, Inc., Farmingdale, New York, USA) according to the manufacturer's instructions.

Histological studies

Hearts, kidneys, lungs and livers were fixed in 4% paraformaldehyde and embedded in paraffin blocks. The heart and kidney sections were stained with Masson trichrome to assess fibrosis (Applied Medical Research Laboratory, Inc., Osaka, Japan). Photomicrographs were analyzed with ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). The area of perivascular fibrosis was calculated as the ratio of the fibrotic area surrounding the vessel to the total vessel area. Heart, kidney, lung and liver sections were stained with hematoxylin–eosin to assess the safety of the vaccine. Samples were observed using an FSX1000 microscope (Olympus, Tokyo, Japan).

Quantitative reverse transcription-PCR

The mRNA levels of collagen type I, collagen type III and transforming growth factor-β (TGF-β) were quantified using quantitative reverse transcription-PCR. Total RNA was extracted from a heart or kidney using the RNeasy Fibrous Tissue Kit (Qiagen Inc., Hilden, Germany). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biomedical Inc., Crown Point, Indiana, USA). Relative gene copy numbers were quantified with PCR using TaqMan Gene Expression Assays (GAPDH: Mm99999915; collagen type I: Mm00801606; collagen type III: Mm01254476; TGF-β: Mm01178820; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). The absolute number of gene copies was normalized to the GAPDH gene copy number and normalized using a standard curve.

Purified antibodies specific for angiotensin II

Affinity-purified antibodies specific for Ang II from rabbit serum were synthesized by the Peptide Institute, Inc. (Osaka, Japan). Briefly, the anti-Ang II antibody titer was measured in rabbits following four times injections of Ang II vaccine with Freund's adjuvant at 2-week intervals. After that, the antibody was purified by exposure the affinity-column purification (cyanogen bromide-activated Sepharose 4B; GE Healthcare).

Neutralizing angiotensin II-induced extracellular signal-regulated kinase phosphorylation

The details of the Western blot analysis were described previously [4]. Briefly, rat vascular smooth muscle cells (VSMCs) were purchased from KAC Co., Ltd. (Kyoto, Japan). The cells were cultured in Roswell Park Memorial Institute 1640 medium from Nacalai Tesque, Inc. (Kyoto, Japan). The cells were cultured at 37 °C in 5% CO_2. Cells were stimulated with Ang II (10^{-7} mol/l) after preincubation of recombinant Ang II with purified antibodies or control rabbit IgG (normal rabbit IgG, Thermo Fisher Scientific, Inc., Rockford, Illinois, USA) for 1 h at 37 °C. Total protein was extracted from the cells using lysis buffer, size-fractionated using SDS–PAGE, and transferred to an Immobilon-P membrane (Merck KGaA, Inc., Darmstadt, Germany). The membrane was incubated overnight with an antibody against extracellular signal-regulated kinase (ERK) or ERK phosphorylation (pERK). The blot was developed using ECL Plus Western blotting detection reagents

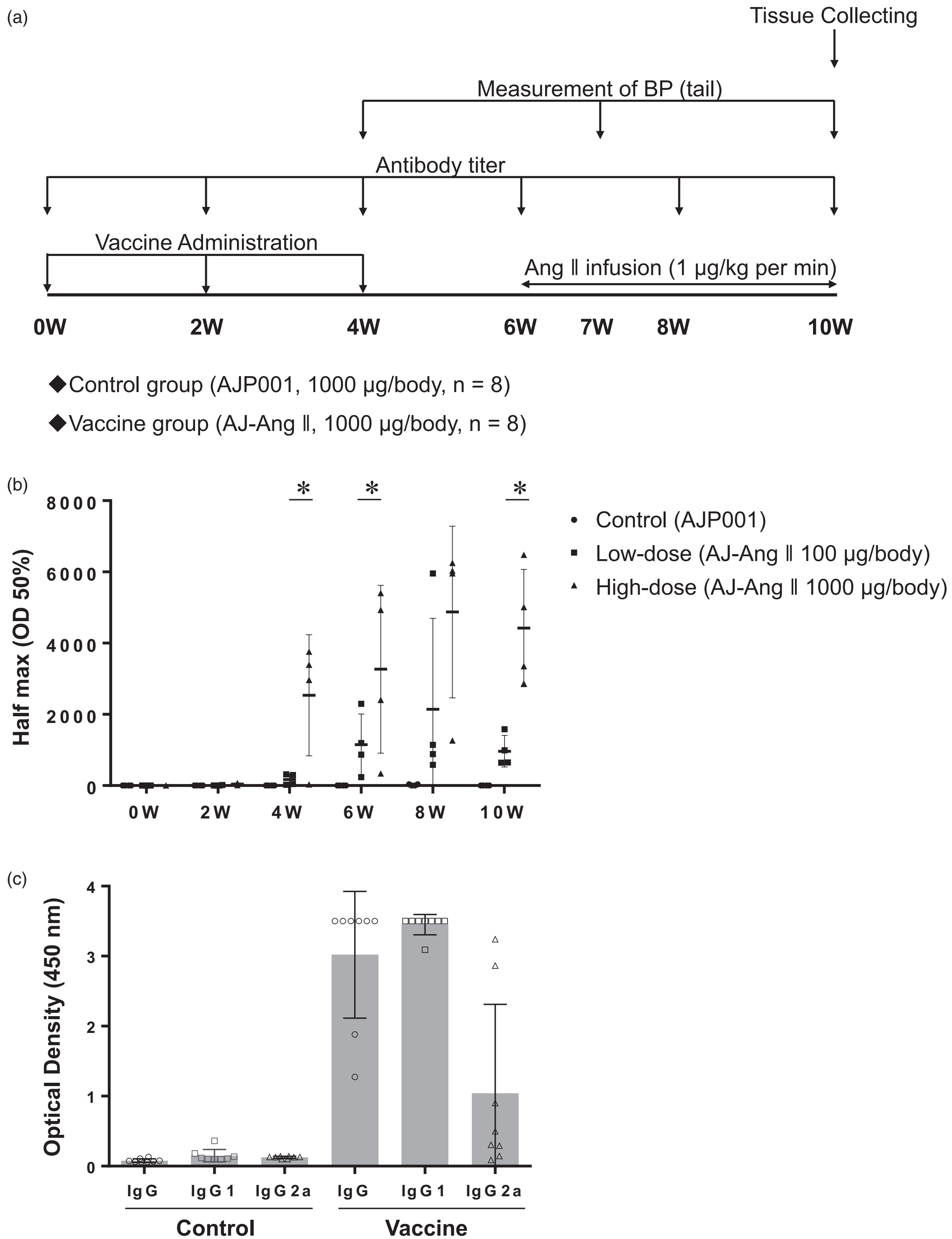


FIGURE 1 Study protocol and evaluation of antiangiotensin II antibodies and T-cell activation. (a) The study protocol used to investigate the efficacy of the conjugate vaccine containing the AJP001 peptide and angiotensin II is shown. AJP001 (control group) or 100 or 1000 μg/mouse AJP001-conjugated peptide vaccine (vaccine groups) ($n = 8$ per group) were injected into 6-week-old male Balb/C mice during weeks 0, 2 and 4. Angiotensin II was infused continuously from weeks 6 to 10. Blood pressure was measured during weeks 4, 7 and 10. (b) Six-week-old male Balb/C mice were divided into high-dose and low-dose vaccine groups (100 or 1000 μg/mouse angiotensin II and AJP001-conjugated vaccine), a control group (1000 μg/mouse AJP001), and a saline group ($N = 4$ per group). The mice were immunized intradermally three times during weeks 0, 2 and 4. The antiangiotensin II antibody titer was measured during weeks 0, 2, 4, 6, 8 and 10. The antibody titer is expressed as the dilution of serum exhibiting half-maximal binding (optical density: 50%) ± SD. $^{*}P < 0.05$ vs. the low-dose group. (c) The total IgG, IgG1 (Th2) and IgG2a (Th1) profiles of the humoral immune response were measured in immunized mice ($n = 8$). Diluted serum samples (1 : 1250) collected during week 8 were quantified by the absorbance at 450 nm. The data are expressed as the mean ± SD.

(Thermo Fisher Scientific, Inc.). The signal levels were visualized using a ChemiDoc Touch imaging system (Bio-Rad Inc.).

Neutralizing angiotensin II-induced nuclear factor-κB promoter activity

The luciferase assay was described previously [4,5]. Briefly, HEK 293 cells were transfected with an nuclear factor (NF)-κB luciferase reporter gene using lipofectamine 2000 transfection reagent. Recombinant Ang II and a purified antibody or control rabbit IgG were preincubated for 1 h at 37 °C. At 24 h after transfection, the cells were stimulated with the preincubated Ang II (10^{-7} mol/l) for 24 h. The promoter activity was normalized to each protein concentration.

Statistical analysis

All values are expressed as the mean ± SD. Differences between two groups were assessed by Student's *t* test. Post-hoc analyses were performed with Tukey's multiple comparison test. *P* values less than 0.05 were considered significant. All statistical analyses were performed using JMP 14.3.0 software (SAS Institute, Cary, North Carolina, USA).

RESULTS

Evaluation of the AJP001-angiotensin II vaccine by the antibody titer and T-cell response

AJ-Ang II or AJP001 (control) was intradermally administered three times during weeks 0, 2 and 4 after the first injection (Fig. 1a). Consistent with previous findings [7], the anti-Ang II antibody titer was significantly increased in the AJP001-Ang II-treated groups after week 4. The antibody titer was significantly higher in the high-dose group (1000 μg/mouse) than in the low-dose group (100 μg/mouse) at weeks 4, 6 and 10 and was sustained for at least 6 weeks after the final injection (Fig. 1b). Therefore, a high dose (1000 μg/mouse) of the AJP001-Ang II vaccine was selected and further evaluated.

We also evaluated the IgG subclass distribution by ELISA using total IgG, IgG1 and IgG2a antibodies. We found that IgG1 was the major antibody isotype in this system (Fig. 1c). Therefore, AJ-Ang II induced a primarily Th2-type response. To evaluate the T-cell response, we measured the production of INF-γ, IL-4 and IL-10 cytokines by splenocytes from mice stimulated with antigens (AJ-Ang II, Ang II or PMA and ionomycin as a positive control) using ELISPOT assays. Stimulation with AJ-Ang II induced the production of IL-4, IL-10, and, to a lesser extent, IFN-γ, whereas Ang II did not induce any cytokines (Fig. 2). These results also suggest that AJP001 has a T-cell epitope that tends to skew the response in the Th2 direction.

Evaluation of antiangiotensin II antibodies induced by AJP001-angiotensin II vaccination

To evaluate the neutralizing function of anti-Ang II antibody induced by AJP001-Ang II vaccine treatment, the polyclonal antibody was obtained and purified from immunized rabbit. The OD (405 nm), which was measurement by

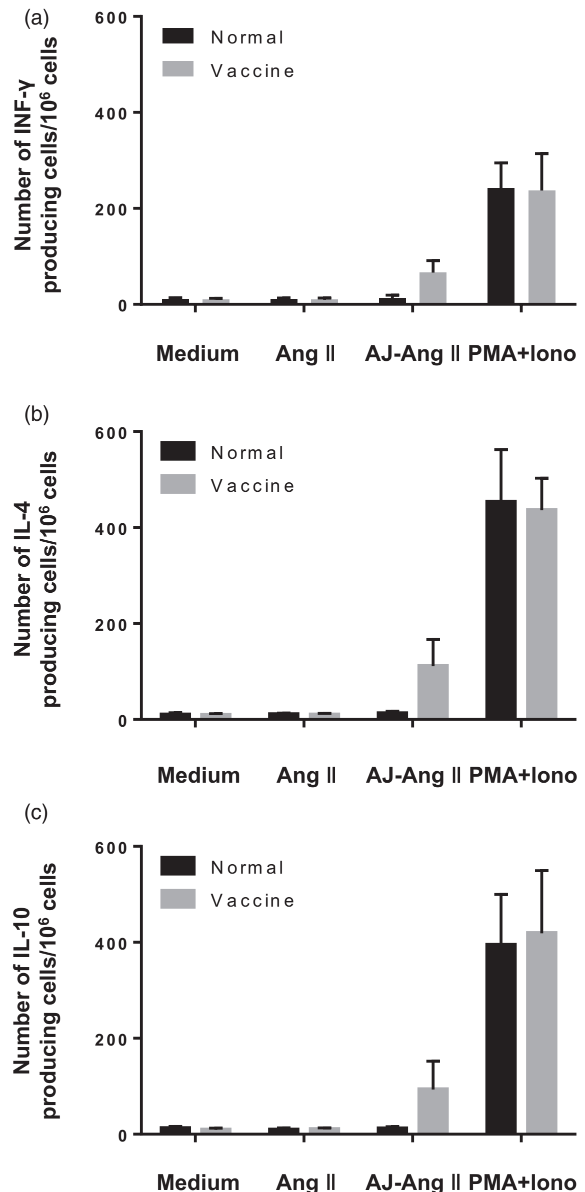


FIGURE 2 Quantification of spots in enzyme-linked immune absorbent spot assays. Enzyme-linked immune absorbent spot assays detected splenocytes that produced (a) INF-γ, (b) IL-4 or (c) IL-10. Splenocytes from mice collected during week 6 were stimulated for 48 h with 10 μg/ml angiotensin II, AJP001-conjugated peptide vaccine or phorbol myristate acetate and ionomycin. The spots in the enzyme-linked immune absorbent spot assay were quantified. The data are expressed as the mean ± SD per 10^6 splenocytes.

ELISA, was elevated gradually after immunization (Supplemental Fig. 1A, http://links.lww.com/HJH/B427). Further, the OD of purified anti-Ang II antibody which was eluted by the affinity-column was higher than that without elution (Supplemental Fig. 1B, http://links.lww.com/HJH/B427).

To evaluate the neutralizing function of the anti-Ang II antibodies induced by AJ-Ang II vaccination, polyclonal antibodies were obtained and purified from immunized rabbits. The concentration of IgG in the purified antibodies was 830 μg/ml, which was measured by ELISA. Although Ang II treatment increased pERK levels at 5 min after

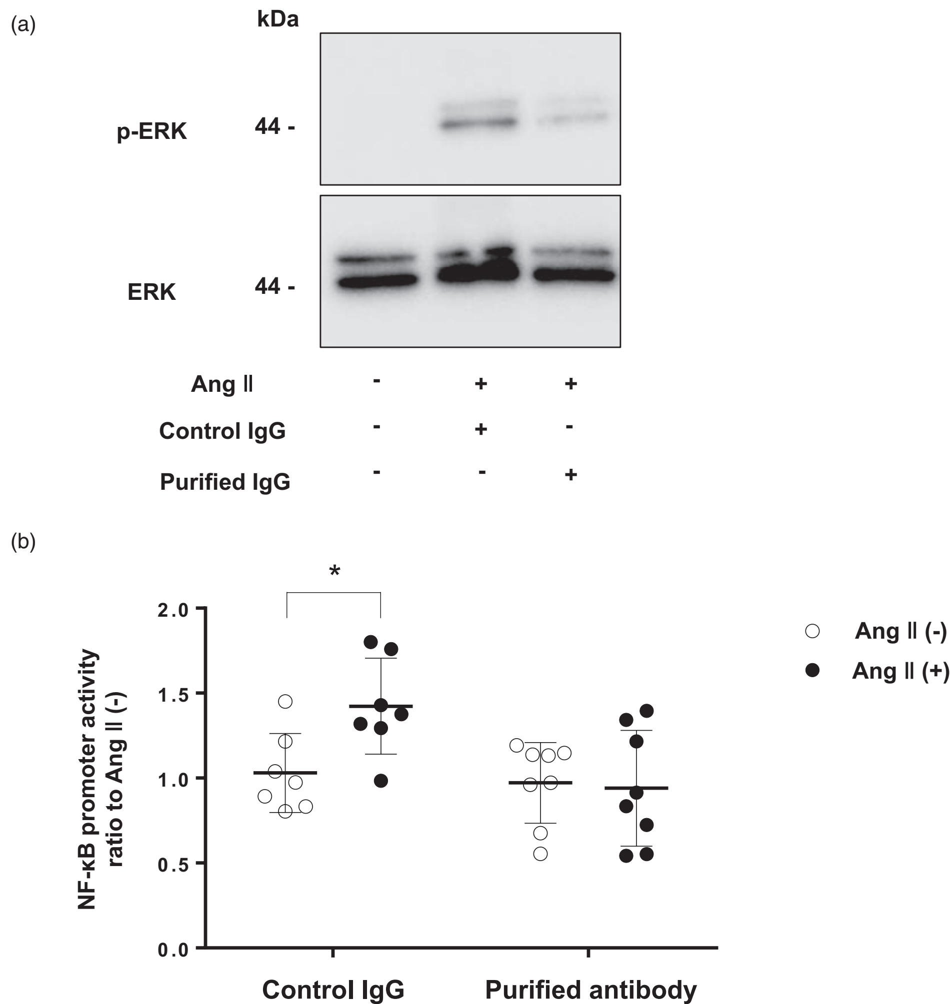


FIGURE 3 Evaluation of neutralizing antibodies induced by angiotensin II and AJP001-conjugated vaccine. (a) Western blotting was used to analyze extracellular signal-regulated kinase 1/2 phosphorylation in vascular smooth muscle cells stimulated with angiotensin II (10^{-7} mol/l) preincubated with control rabbit IgG or purified antibodies specific for angiotensin II for 1 h. (b) The promoter activity of nuclear factor (NF)-κB was assessed by measuring luciferase activity following normalization to each protein concentration. It was evaluated in vascular smooth muscle cells stimulated with angiotensin II (10^{-7} mol/l) preincubated with control rabbit IgG or purified antibodies specific for angiotensin II for 1 h. The data are expressed as the ratio to the corresponding group without angiotensin II ($n = 7$–8). Luciferase activities were increased significantly in the control IgG group but not in the purified antibody group. The data are expressed as the mean ± SD.

treatment in adult human VSMCs, cotreatment with the purified antibodies and Ang II resulted in a decrease in pERK levels in the VSMCs compared with treatment with control IgG following preincubation of the antibody and Ang II (Fig. 3a). In the evaluation of NF-κB promoter activity, the ratio of promoter activity was significantly increased by Ang II treatment (1.60 vs. 1.00, $P = 0.03$), whereas cotreatment with the purified antibodies attenuated the Ang II-induced increase (Fig. 3b).

Efficacy of AJP001-angiotensin II in reducing blood pressure

To confirm the efficacy of AJ-Ang II, SBP was measured in mice with Ang II infusion by the tail-cuff method during week 4 (before Ang II infusion), week 7 (1 week after Ang II infusion) and week 10 (4 weeks after Ang II infusion), as shown in Fig. 4a and b. Although AJ-Ang II or AJP001 was administered during weeks 0, 2 and 4 after the first injection, there was no significant difference in SBP at week 4 (107.1 ± 4.4 vs. 110.7 ± 7.0 mmHg, $P = 0.24$). SBP was elevated 1 week after Ang II infusion in the control group, whereas the SBP in the vaccine group was significantly lower than that in the control group at week 7 (132.0 ± 11.4 vs. 146.9 ± 9.7 mmHg, $P = 0.02$). This tendency was sustained at 4 weeks after Ang II infusion (135.9 ± 8.5 vs. 154.9 ± 16.8 mmHg, $P = 0.02$) (Fig. 4a). Significantly, plasma Ang II concentrations at week 10 were significantly higher in the vaccine group than in the control group (10.5 ± 4.5 vs. 2.8 ± 2.3 ng/ml, $P < 0.05$, Fig. 4b).

Pathological analysis of the effects of AJP001-angiotensin II on the heart and other organs

Ang II infusion-induced cardiac interstitial and perivascular fibrosis in the control group at week 10 (4 weeks after Ang II infusion), whereas the mice in the vaccine group showed fewer fibrotic changes (Fig. 5a). Quantification of the

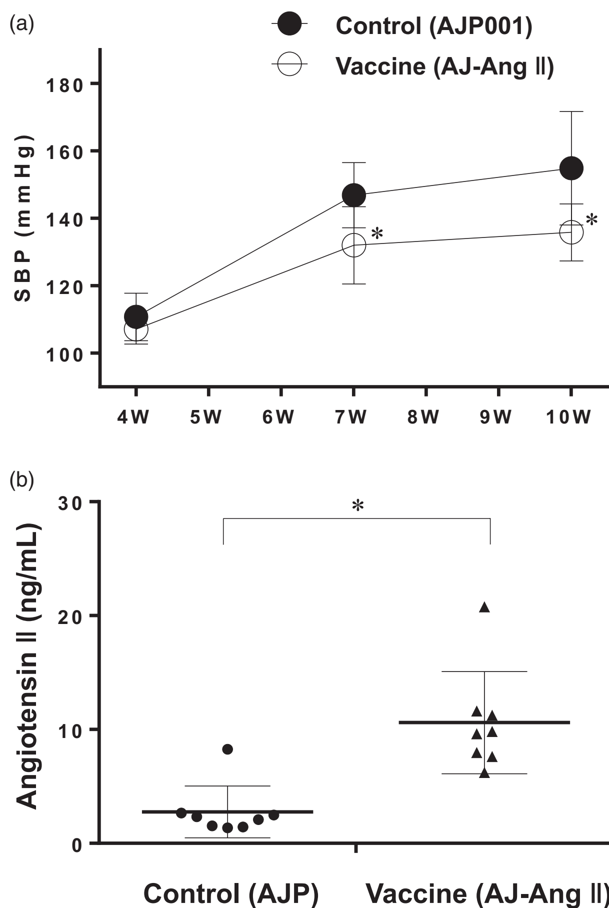


FIGURE 4 Effects of the vaccine on SBP and the plasma angiotensin II concentration in mice treated with angiotensin II infusion. (a) SBP was measured in mice treated with AJP001 (control; $N=8$) or AJP001-angiotensin II (vaccine; $N=8$) during weeks 4, 7 and 10. Data are expressed as the average of each group. (b) The plasma angiotensin II concentration at week 10 is shown. All data are expressed as the mean ± SD. $^{*}P<0.05$ vs. the control group.

perivascular fibrosis area indicated that perivascular fibrosis was significantly decreased in the vaccine group compared with the control group (Fig. 5b). Furthermore, we evaluated the mRNA levels of markers of fibrosis, including collagen type I, collagen type III and TGF-β, in the heart and kidneys. In the vaccine group, the levels were significantly lower than those in the control group (Fig. 5c and d).

We also evaluated histological findings for the heart, kidneys, lungs and liver in mice treated with AJ-Ang II or AJP001. There was no abnormal accumulation of inflammatory cells and no obvious changes between the mice in the control or vaccine group and normal mice (without Ang II infusion and vaccination) (Fig. 6).

DISCUSSION

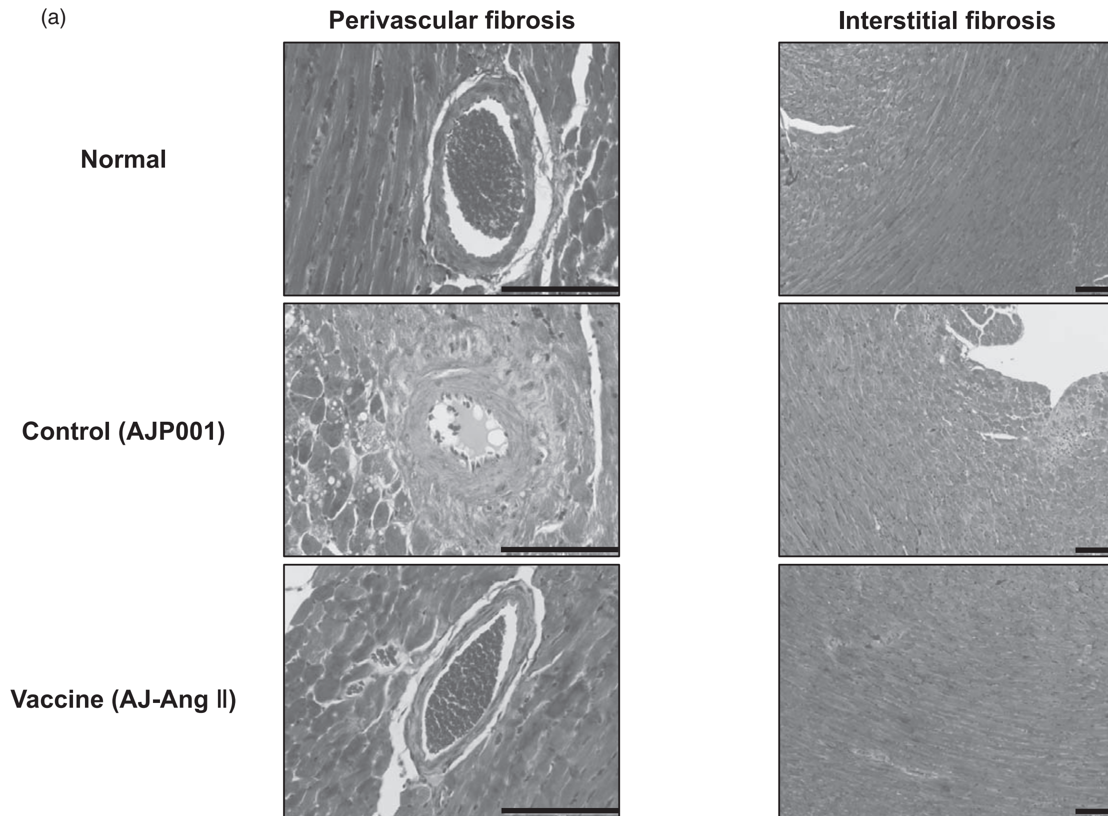
In the current study, we demonstrated the impact of AJ-Ang II without an adjuvant on the reduction in BP in mice treated with Ang II infusion.

When we design B-cell-type peptide vaccines for chronic diseases, a large carrier protein is required to activate T cells via antigen presentation, leading to B-cell activation [8]. However, in terms of clinical application, large carrier proteins have several problems as a drug formulation, including provoking undesirable immune responses. To simplify the drug formulation and avoid using a large carrier protein, we utilized a novel peptide, AJP001, that possessed both a T-cell epitope and an adjuvant action, and its direct conjugation to Ang II was effective in this study. Since AJ-Ang II can be synthesized as a single peptide, it has an advantage in drug manufacturing compared with other types of vaccines. Importantly, AJP001 possessed strong activity in inducing the upregulation of CD86 and CD54 expression and activating the inflammasome and NF-κB pathway, leading to adjuvant activity [7]. In this study, IgG subtype analysis showed that IgG1, which is a marker of Th2 lymphocytes [9], was the major antibody isotype (Fig. 1c), and an ELISPOT assay showed increased production of IL-4 and IL-10, which are Th2 cytokines [10], by analysis of splenocytes stimulated with AJ-Ang II (Fig. 2). These results suggested that the adjuvant action of AJP001 was partially similar to that of alum adjuvants in skewing the response in the Th2 direction, which might be better for inducing neutralizing antibodies without antibody-dependent cytotoxicity. Thus, AJ-Ang II successfully induced a humoral immune response without the addition of any adjuvants in mice.

In terms of translational research, it is extremely important to establish the safety of this vaccine targeting self-antigens. In pathological analysis, we found no obvious differences (i.e. accumulation of immune cells) in the heart, kidneys, lungs and liver between normal and immunized mice (Fig. 6). In addition, in the ELISPOT assay, the production of Th1/Th2 cytokines was not observed in splenocytes after stimulation with Ang II, suggesting that Ang II treatment alone (without conjugation to AJP001) does not activate T cells. Furthermore, we never observed a 'booster effect' of the antibody in AJ-Ang II following Ang II infusion (data not shown). These results suggested that the immune reaction was provoked by only AJ-Ang II treatment, not endogenous Ang II. Thus, autoimmune responses to endogenous Ang II can be avoided in mice.

Significantly, plasma Ang II concentrations were significantly higher in the vaccine group than in the control group (Fig. 2b), which is consistent with previous findings [11,12]. The major cause of this result may be feedback via blockade of the renin–Ang system by anti-Ang II antibodies. Indeed, we found that neutralizing Ang II-induced pERK downstream of AT1R signaling by western blot analysis (Fig. 3a and b). Even though the plasma Ang II concentration was increased in the vaccine group, Ang II-induced BP elevations and fibrosis in the perivascular areas in the heart were significantly decreased in the vaccine group compared with the control group. These results suggest that the induced anti-Ang II antibodies successfully attenuate the Ang II signal in the systemic and local renin–Ang systems. It is widely known that Ang II induces not only hypertension but also hypertrophic and fibrotic reactions [13–15]. Thus, AJ-Ang II treatment might be useful for attenuating the tissue renin–Ang system.

There are several limitations in this investigation. First, as mentioned above, although we confirmed the predominance of humoral immune response and B-cell activation following vaccination using ELISPOT assay or IgG subclass



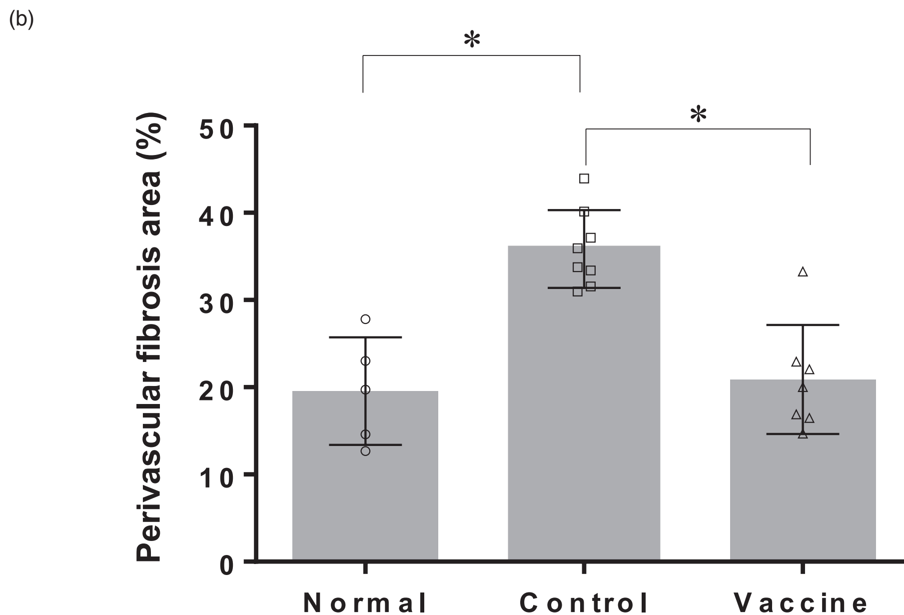


FIGURE 5 Effects of the angiotensin II and AJP001-conjugated vaccine on fibrosis and histological changes. (a) A representative photomicrograph of Masson trichrome staining of the heart. Cardiac interstitial and perivascular fibrosis were monitored in the control and vaccine groups following angiotensin II infusion. Scale bar, 100 μm. (b) Quantitation of the perivascular fibrosis area ($n = 5$–7 per group). (c and d) The mRNA levels of collagen I, collagen III and transforming growth factor-β determined using reverse transcription-PCR analysis of the heart (c) and kidneys (d) ($n = 5$ per group). The results are expressed as the ratio of gene expression in control (AJP001) mice or vaccine (angiotensin II and AJP001-conjugated vaccine 1000 μg/body) mice to that in normal mice. All data are expressed as the mean ± SD. *$P < 0.05$ vs. the control group.

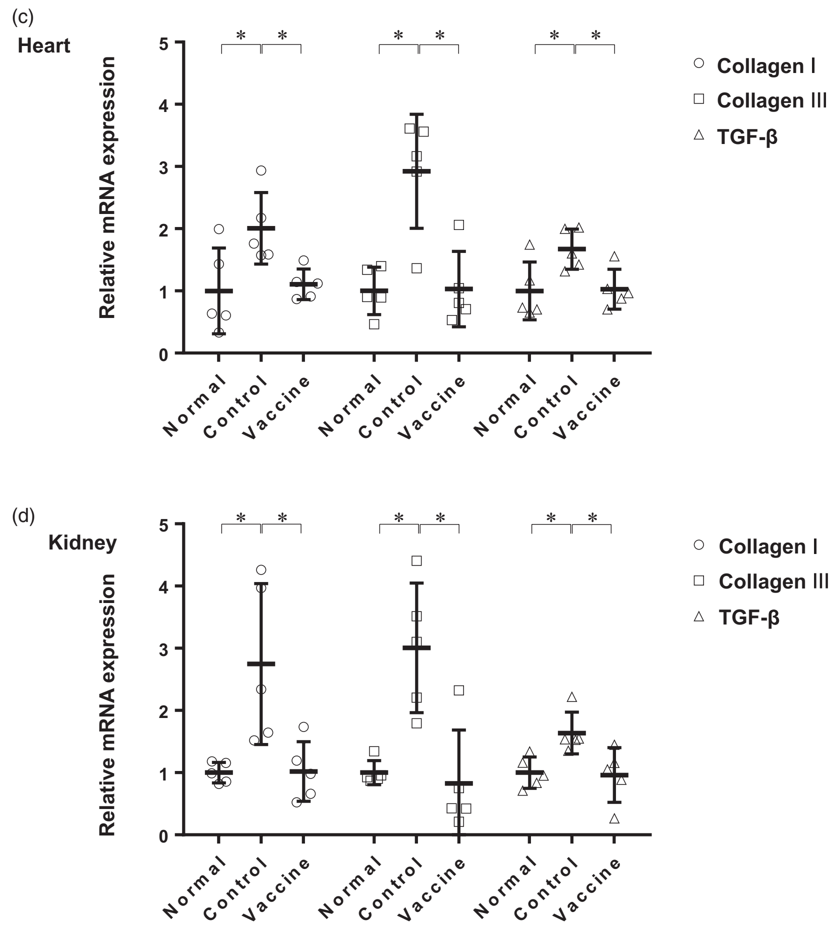


FIGURE 5 (Continued).

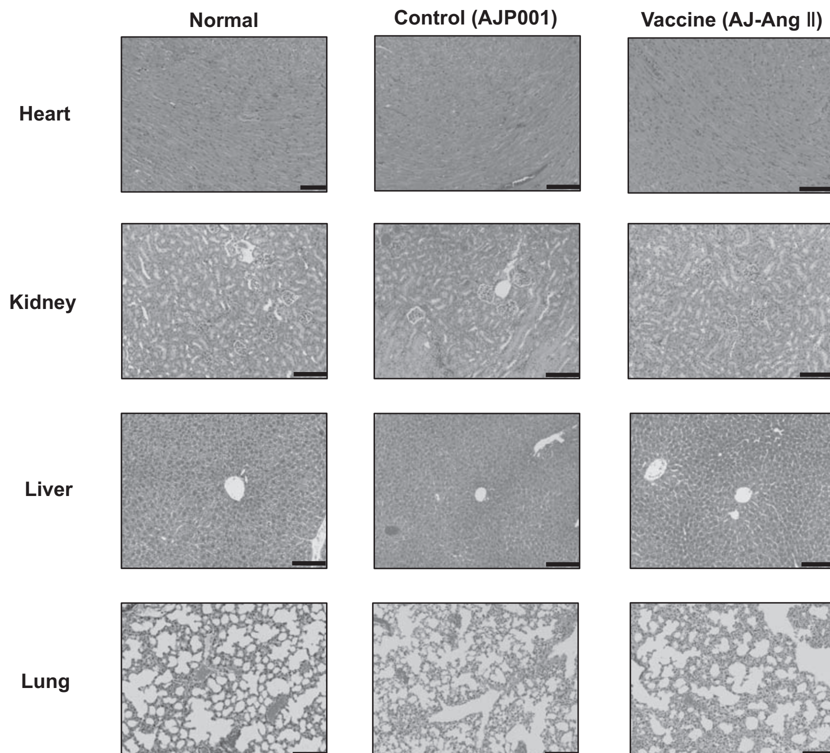


FIGURE 6 Representative photomicrographs of hematoxylin–eosin staining of the heart, kidneys, lungs and liver. Scale bar, 100 μm.

analysis in this study, we cannot completely exclude the activation of cytotoxic T-cell or inflammatory cytokines in the long terms. Second, since renin–Ang system may be activated to react to the salt and volume depletion or cardiac shock, the long-term blockade by anti-Ang II antibodies is disadvantage in case of these emergencies. However, we have some therapeutic options against hypotension, including the administration of salt or catecholamine. In terms of difference among individuals of renin–Ang system, the response to its blockade is different among age or race which usually depends on salt-sensitivity [16–18]. These variations may affect the efficacy of Ang II vaccine against hypertension. Taken together, regarding the clinical application of vaccines against hypertension, several tasks should be addressed for the concerns of safety and efficacy by further investigations.

Here, we demonstrated the efficacy and safety of a novel peptide vaccine, AJ-Ang II, against hypertension in mice with Ang II infusion. In an aging society, therapeutic vaccines play a key role in overcoming hypertension because medication nonadherence is one of the major causes of uncontrolled BP [1–3]. Since AJ-Ang II has advantages in drug manufacturing and formulation, we hope that the development of a therapeutic vaccine based on our system will be realized in the future.

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Conflicts of interest

The Department of Health Development and Medicine is an endowed department supported by AnGes, Daicel and FunPep. The Department of Clinical Gene Therapy is financially supported by AnGes, Shionogi, Boeringher, Fancl, Saisei Mirai Clinics, Rohto and FunPep. R.M. is a stockholder of FunPep and AnGes. A.T. is an employee and stockholder of FunPep. The funder (FunPep) provided support in the form of salaries for authors (A.T.) but did not have any additional role in the study design, data collection and analysis, decision to publish or preparation of the article. All other authors declare no competing interests.

REFERENCES

1. Burnier M, Egan BM. Adherence in hypertension. *Circ Res* 2019; 124:1124–1140.
2. Vrijens B, Vincze G, Kristanto P, Urquhart J, Burnier M. Adherence to prescribed antihypertensive drug treatments: longitudinal study of electronically compiled dosing histories. *BMJ* 2008; 336:1114–1117.
3. Hill MN, Miller NH, Degeest S, Materson BJ, Black HR, Izzo JL Jr, *et al.*, American Society of Hypertension Writing Group. Adherence and persistence with taking medication to control high blood pressure. *J Am Soc Hypertens* 2011; 5:56–63.
4. Nakagami F, Koriyama H, Nakagami H, Osako MK, Shimamura M, Kyutoku M, *et al.* Decrease in blood pressure and regression of cardiovascular complications by angiotensin II vaccine in mice. *PLoS One* 2013; 8:e60493.
5. Koriyama H, Nakagami H, Nakagami F, Osako MK, Kyutoku M, Shimamura M, *et al.* Long-term reduction of high blood pressure by angiotensin II DNA vaccine in spontaneously hypertensive rats. *Hypertension* 2015; 66:167–174.
6. Nakagami H, Morishita R. Recent advances in therapeutic vaccines to treat hypertension. *Hypertension* 2018; 72:1031–1036.
7. Tenma A, Nakagami H, Tomioka H, Sakaguchi M, Ide R, Koriyama H, *et al.* AJP001, a novel helper T-cell epitope, induces a humoral immune response with activation of innate immunity when included in a peptide vaccine. *FASEB Bioadv* 2019; 1:760–772.
8. O'Hagan DT, Fox CB. New generation adjuvants – from empiricism to rational design. *Vaccine* 2015; 33:B14–B20.
9. Finkelman FD, Holmes J, Katona IM, Urban JF Jr, Beckmann MP, Park LS, *et al.* Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol* 1990; 8:303–333.
10. Snapper CM, Paul WE. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987; 236:944–947.
11. Ambühl PM, Tissot AC, Fulurija A, Maurer P, Nussberger J, Sabat R, *et al.* A vaccine for hypertension based on virus-like particles: preclinical efficacy and phase I safety and immunogenicity. *J Hypertens* 2007; 25:63–72.
12. Wakayama K, Shimamura M, Suzuki JI, Watanabe R, Koriyama H, Akazawa H, *et al.* Angiotensin II peptide vaccine protects ischemic brain through reducing oxidative stress. *Stroke* 2017; 48:1362–1368.
13. Baker KM, Booz GW, Dostal DE. Cardiac actions of angiotensin II: role of an intracardiac renin–angiotensin system. *Annu Rev Physiol* 1992; 54:227–241.
14. Balakumar P, Jagadeesh G. A century old renin–angiotensin system still grows with endless possibilities: AT1 receptor signaling cascades in cardiovascular physiopathology. *Cell Signal* 2014; 26:2147–2160.
15. Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol Rev* 2000; 52:11–34.
16. Weidmann P, Beretta-Piccoli C, Ziegler WH, Keusch G, Glück Z, Reubi FC. Age versus urinary sodium for judging renin, aldosterone, and catecholamine levels: studies in normal subjects and patients with essential hypertension. *Kidney Int* 1978; 14:619–628.
17. He J, Klag MJ, Appel LJ, Charleston J, Whelton PK. The renin–angiotensin system and blood pressure: differences between blacks and whites. *Am J Hypertens* 1999; 12:555–562.
18. Saunders E, Gavin JR 3rd. Blockade of the renin–angiotensin system in African Americans with hypertension and cardiovascular disease. *J Clin Hypertens (Greenwich)* 2003; 5:12–17.