Article

Vascular Damages in Rats Immunized by α_1-Adrenoceptor Peptides

Zihua Zhou[1], Yuhua Liao[1, 2], Liudong Li[1], Fen Wei[1], Bin Wang[1], Yumiao Wei[1], Min Wang[1] and Xiang Cheng[1]

Autoantibodies against the α_1-adrenoceptor which had agonist activity as norepinephrine might play roles in the progression of hypertension, but whether the autoantibodies could induce vascular remodeling as norepinephrine is not clear. In this paper, the models with antibodies against the α_1-adrenoceptor were made by immunizing Wistar rats with the synthesized the second extracellular loop of α_1-adrenoceptor peptides. The homo-age male Wistar rats received BSA in the same immunizing manner and male spontaneous hypertensive rats (SHR) were used as control. All the rats were raised for one year. The blood pressure and morphological changes of arteries were measured. In the end, despite the systolic blood pressure of immunized rats had no difference with normal control, the media thickness of aortas and ratio of media to lumen in the third-order arteries of mesenteric vasculature were increased in immunized rats. The observation with electron microscope showed that the mitochondria of vascular smooth muscle cells (VSMCs) had notable hyperplasia, and the interstitial collagen fibril was increased too. The effects of purified antibodies against α_1-adrenoceptor on the proliferation of cultured VSMCs, and the expressions of c-jun, c-fos and α_1-adrenoceptor were detected. The results showed that the antibodies could promote the proliferation of cultured VSMCs, and enhance the expression of c-jun both *in vitro* and *in vivo*. So we concluded that antibodies against the α_1-adrenoceptor could contribute to vascular damages in rats by stimulating the growth of VSMCs which might be caused by the increased c-jun expression, and might play particular roles in the pathological changes of hypertension. *Cellular & Molecular Immunology*. 2008;5(5):349-356.

Key Words: α_1-adrenoceptor, autoantibody, vascular remodeling

Introduction

The immune system, especially autoantibodies with agonist-like activity, plays important roles in the hypertension (1, 2). Autoantibodies against the α_1-adrenoceptor with agonist-like activity had been found in patients with malignant hypertension, primary hypertension and refractory hypertension (3-6). The antibodies could be found in 25.3% of primary hypertensive patients and 36.7% of refractory hypertensive patients (6). Like phenylephrine, these autoantibodies could increase the beating frequency of neonatal cultured rat cardiomyocytes and modulate intracellular Ca^{2+} *via* L-type calcium channels (7). They also had modulatory effects on heart mast cells (8). These functions could be blocked by the α_1-adrenoceptor antagonist prazosin (3-5). These studies suggested the autoantibodies could contribute the progression of hypertension, just like the sympathetic nervous system in hypertensive patients.

Previous reports identified that treatments with norepinephrine (NE) could induce cardiac remodeling in rats (9, 10), and increased sympathetic stimulation of the arteries might contribute to the arterial remodeling, which would be induced by α_1-adrenoceptor-dependent proliferation, hypertrophy, and migration of vascular smooth muscle cells (VSMCs) and adventitial fibroblasts (11, 12). These findings suggest that NE may contribute to adverse structural remodeling in hypertension. But, whether the autoantibodies against α_1-adrenoceptor have these roles in hypertension is not clear.

In our earlier research, cardiac remodeling could be induced by antibodies against the α_1-adrenergic receptor in Wistar rat model which were immunized by synthesized the second extracellular loop of α_1-adrenoceptor peptides (13). In a clinical investigation including 553 hypertensive patients, we found the ratios of left atria enlargement and left ventricular enlargement in the group with the autoantibodies against α_1-adrenoceptor were significantly higher than those in patients without these autoantibodies (53.6% *vs* 44.3%, *p*

[1]Institute of Cardiology, Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430022, China;

[2]Corresponding to: Dr. Yuhua Liao, Institute of Cardiology, Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430022, China. Tel: +86-27-8572-6376, Fax: +86-27-8572-7340, E-mail: liaoyh27@163.com

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< 0.05) (14). The results suggest the autoantibodies could induce cardiac remodeling like NE, but the effects of the autoantibodies on the pathological structure of arteries had not been well-known in hypertension.

Previous studies have identified chronic α_1-adrenoceptor stimulation induced VSMC growth *in vitro* and *in situ* (15-17). In this paper, we hypothesized that autoantibodies against the α_1-adrenoceptor had the same effects with NE, and they could induce vascular remodeling in rats immunized by peptides of the 2nd extracellular loop of α_1-adrenoceptor.

Materials and Methods

Animal model

The methods were similar as previously described (13, 18). Male Wistar rats aged 5 weeks were immunized with the synthesized peptides of the second extracellular loop of α_{1A}-adrenoceptor at weeks 0, 2, 4, 6, 8. The peptides (residues 192 to 218: Gly-Trp-Lys-Glu-Pro-Val-Pro-Pro-Asp-Glu-Arg-Phe-Cys-Gly-Ile-Thr-Glu-Glu-Ala-Gly-Tyr-Ala-Val-Phe-Ser-Ser-Val) were synthesized by the type PSSM-8 PEPTIDE SYNTHESIZER (Shimadzu company, Japan). Rats were immunized by subcutaneous injection with 300 μg peptides coupled with bovine sera albumin (BSA) and the same volume of complete or incomplete Freund's adjuvant. The homo-age male Wistar rats received BSA in the same manner were used as normal control, and homo-age male spontaneous hypertensive rats (SHR) were used as hypertensive control. All the rats were raised for one year. The sera were collected at the first and every one or two months after immunization. All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Authority for Laboratory Animal Care.

Blood pressure and the titer of the antibodies

Systolic blood pressure (SBP) was measured with a computerized tail-cuff system before immunization and each one or two months after immunization. Antibodies against the α_1-adrenoceptor were detected by ELISA as described (13). The titer was shown by the value of P/N when the sera were diluted at 1:40 [(P/N = (A value of test - A value of blank)/(A value of negative control - A value of blank)].

Tissue preparation and vascular morphometry

The rats were sacrificed after one year post-immunization and perfusion-fixed. The ascending aorta and the third-order branch of mesenteric arterial tree were excised. The fixed transverse sections of ascending aorta and the third-order branch of mesenteric arteries were embedded in paraffin, cut into 5-μm cross sections, and stained with collagen-specific van Gieson or hematoxylin-eosin. The pictures were collected by an automated image processor (×10 magnification) connected with an Olympus microscope (×10 magnification lens). The media thickness, medial cross-section area, and lumen diameter of arteries were measured, and the ratio of media thickness to lumen diameter was calculated. Four sectors of each sample were analyzed.

Detection of ultrastructural change of aorta by electron microscope

Two rats in each group were killed by decapitation after anaesthesia. The ascending aorta was rinsed for 1 min in 0.01 mol/L cold PBS (pH 7.4), and then part of ascending aorta was fixed immediately in 0.25% glutaraldehyde for transmission electron microscopic analysis.

Immunohistochemistry

The aortas were prepared using generally methods. Immunostaining was carried out using streptavidin/biotin immunoperoxidase method. Specific c-jun or c-fos primary antibodies were appropriately diluted at 1:100. Sections were reacted with 3,3'-diaminobenzidine as the chromogen, counterstained with hematoxylin, and examined by light microscopy.

Preparation of immunoglobulin

The antibodies against α_1-adrenoceptor were purified from the immunized rats by the immune affinity chromatography method (19). In brief, immunoglobulin fractions (IgG) were loaded on a Sepharose 4B CNBr-activated gel (Pharmacia, USA), to which the peptides corresponding to the second extracellular loop of human α_1-adrenoceptor were covalently linked. The antibodies were eluted with 3 mol/L potassium thiocyanate (pH 7.4) followed by immediate super-filtration by millipore Centriplus 50000, changed and concentrated the buffer with PBS. Immunoglobulin fractions from normal Wistar rats were used as negative control.

Cell culture

VSMCs from thoracic aorta of male Wistar rats weighing between 200 g and 250 g were isolated and cultured in DMEM supplemented with 10% newborn bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, kept in a humidified incubator at 37°C in 5% CO_2, and split one or two times a week at 1:2 with the use of a trypsin-EDTA solution. Cells were characterized by immunohistochemical assay with anti-α-actin mAbs (Santa Cruz Biotechnology, USA): 90% of the cells expressed α-actin. Cells between passages 5 and 8 were used in these experiments. Before the addition of the drugs, the medium were replaced by DMEM containing 0.4% newborn bovine sera for 36 h.

Cell proliferation

The proliferation of VSMCs was determined by DNA synthesis and cell cycle analysis. DNA synthesis was measured by ELISA using BrdU cell proliferation assay kit (Oncogene, USA) according to the protocol of the manufacturer. In brief, VSMCs were plated into 96-well plates at a density of 1×10^5 cells/ml, 0.1 ml/well, in complete DMEM growth medium and allowed to grow until they reached 70% confluence. The medium was replaced with DMEM containing 0.4% newborn bovine sera for 36 h. Then the medium were replaced by the DMEM containing 0.4% newborn bovine sera, and the antibodies against α_1-adrenoceptor (1:40) and

Table 1. Primer sequences and PCR conditions

Primers	Cycles	Annealing (°C)	Length (bp)	Sequences	
				Upper	Lower
GAPDH	33	56.8	516	5'-AATGCATCCTGCACCACCAA-3'	5'-GTAGCCATATTCATTGTCATA-3'
c-fos	40	54.4	80	5'-GGACAGCCTTTCCTACTACCATT-3'	5'-CGCAAAAGTCCTGTGTGTTGA-3'
c-jun	32	53.2	290	5'-TAGTCCCTCCCGTCTGGTTG-3'	5'-TCTAGGAGTCGTCAGAATCC-3'
α_{1A}-receptor	45	50.6	204	5'-CGAGTCTACGTAGTAGCC-3'	5'-GTCTTGGCAGCTTTCTTC-3'
α_{1B}-receptor	45	50.2	201	5'-ATCGTGGCCAAGAGGACC-3'	5'-TTTGGCTGCTTTCTTTTC-3'
α_{1D}-receptor	35	50	218	5'-CGCGTGTACGTGGTCGCAC-3'	5'-CTTGGCAGCCTTTTTC-3'

various drugs were added individually. The inhibitors were added into the wells 1 h before the addition of the antibodies. VSMCs were cultured for 12 h, 24 h or 48 h, and BrdU was added for an additional 12-h incubation period before fixation. After washed and fixed, the plates were incubated with mouse anti-BrdU antibody for 1 h at room temperature. Then horseradish peroxidase-conjugated goat anti-mouse IgG was added and incubated for 30 min at room temperature. After washed, the substrate solution and stop solution were added in turn, and the absorbance was measured at dual wave lengths of 450/595 nm. The blank control, background control and base control were set up, and the value was expressed by the ratio of OD of the treated samples / OD of the base control samples.

Cell cycle distribution was observed by flow cytometry using propidium iodide (PI) for DNA staining. Cells were trypsinized in 1 ml of trypsin/EDTA for at least 5 min, and then the reaction was stopped by the addition of 1 ml of serum-containing DMEM. The samples were centrifuged at 1,000 rpm for 5 min, washed in ice-cold PBS, and fixed in 70% cold ethanol. The washed pellet was then resuspended in 1 ml PBS into which 100 μg/ml of RNase A was added to remove interfering double stranded RNA, and 5 μg/ml PI was added to stain DNA. Cells were incubated at 37°C for 10-15 min in the dark. DNA content of cells was analyzed by FACSCalibur using CellQuest software (Becton-Dickinson, USA). The percentages of cells in various stages of the cell cycle were determined using "multi-cycle" program (ModFit LT software).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA from VSMCs was prepared using Trizol reagent (Invitrogen, USA). RT-PCR was performed with the OneStep RT-PCR kit (TakaRa, Japan) according to the manufacturer's procedure. Primer sequences and major PCR conditions were listed in Table 1. Each PCR product was size-fractionated by 2% agarose gel electrophoresis. The gel was stained with ethidium bromide (1 μg/ml), visualized by an ultraviolet transilluminator, and photographed. The density of each PCR band was measured and analyzed by ImageMaster VDS analysis software (Pharmacia Biotech), and the amount of mRNA products was expressed as the ratio to GAPDH mRNA product, which served as the internal control.

Western blotting

Total protein was extracted from VSMCs using homogenization buffer (50 mmol/L Tris-HCI, pH 7.4, 150 mM NaCl, 10% vol/vol glycerol, 10 mmol/L EDTA, 0.1% SDS, 1% Triton X-100, 1 mM PMSF, 5 μg/ml aprotinin, 5 μg/ml pepstatin, 5 μg/ml leupeptin) and the protein concentration was determined by Bradford method using BSA as the standard. For Western immunoblotting detection, 50 μg of total protein was resolved on 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Invitrogen, USA). c-jun, c-fos and β-actin primary antibodies were all used at a dilution of 1:400 (Santa Cruz Biotechnology, USA). Protein bands were visualized using the enhanced chemiluminescence detection system (Pierce, USA). Quantification of the protein bands was carried out using laser densitometry. Equality of protein loading on membranes and complete transfer were checked by staining gels and membranes with Coomassie Blue. All Western immunoblots were performed at least three times.

Statistical analyses

Results are expressed as mean ± standard deviation. The statistical significance of differences between the groups was determined using Student's *t* test by SPSS 10.0. The $p < 0.05$ was considered significant.

Results

SBP and titer of antibodies against α_1-adrenoceptor in rat model

The antibodies began to be produced after two weeks post-immunization. The titer peaked at the 3rd month, and then fell slowly. But the titer was still very high (at least 1:1,280) in one year after immunization. In normal control rats, the antibodies could not been found in all course. But in SHR group, the antibodies were found in 1 rat (1:40) at the end point that had no antibodies at the beginning, despite the titer was sharply lower than that of the immunized group. The SBP of the immunized rats was in all times higher than that before immunization, while SBP and HR of immunized rats had no significant differences compared with those of the normal control, and were all markedly lower than those of the SHR group which had no difference at first. The change

Table 2. The SBP and HR among SHR, immunized group and normal control

	Treatment	Time (months)							
		0	1	2	3	5	7	9	12
SBP	SHR	113.9 ± 7.6	171.7 ± 19.3*	182.2 ± 14.0*	188.5 ± 18.7*	186.1 ± 13.0*	191.0 ± 13.6*	200.1 ± 21.2*	173.0 ± 32.3*
(mmHg)	Immunized	103.5 ± 6.9	110.3 ± 7.5	112.0 ± 7.1	109.2 ± 6.8	110.6 ± 6.2	117.9 ± 5.9	118.6 ± 6.6	115.3 ± 8.3
	Normal	102.7 ± 7.5	113.3 ± 7.3	114.5 ± 5.3	113.1 ± 7.3	106.6 ± 5.5	120.3 ± 7.1	109.2 ± 9.5	118.2 ± 7.9
HR	SHR	396 ± 33	409 ± 33*	406 ± 17*	423 ± 23*	431 ± 26*	419 ± 38*	404 ± 28*	420 ± 27*
(beat/min)	Immunized	433 ± 16	378 ± 30	359 ± 20	359 ± 32	361 ± 31	354 ± 30	360 ± 34	393 ± 33
	Normal	396 ± 37	368 ± 28	366 ± 26	363 ± 35	356 ± 27	374 ± 31	347 ± 29	376 ± 22

*Compared to immunized group and normal control, $p < 0.01$.

of SBP with time was shown in Table 2.

Structural change of arteries in immunized rats

The pathological changes of arteries caused by the antibodies were observed. The data of vascular morphometry were summarized in Table 3. Compared to the normal control, the media of the third-order branch of the mesenteric arteries in immunized rats became thicker, the lumen became smaller, and the media/lumen ratio was significantly larger than that of the normal. The media thickness of aortas in immunized rats was larger than that of the normal rats too, although the data were smaller than that of the SHR. These results suggested the growth and/or collagen secretion of VCMCs were increased in immunized rats (Figure 1). Interestingly, early atherosclerosis plaques were found in the aortas of immunized rats (data not shown), but not observed in normal control, nor in SHR group. The functions of the autoantibodies on atherosclerosis need to be studied further.

To ensure the change observed with light microscope, the ultrastructural changes of arteries were studied with electron microscopy. In the aortas of the immunized rats, the mitochondria of VSMCs had notable hyperplasia. The energy of the cells was provided by the mitochondria, so above result suggested the contraction and/or synthesis of VSMCs were improved. Another obvious ultrastructural change of the aortas was that the interstitial collagen fibril was increased (Figure 2), in accordance with the results of the light microscope.

Induction of the proliferation of cultured rat VSMCs by antibodies against the α_1-adrenoceptor

Previous reports identified that activation of α_1-adrenoceptor in rat myocytes and VSMCs stimulated DNA synthesis, protein synthesis, and growth-related gene expression (17, 20). We wondered if antibodies against the α_1-adrenoceptor could stimulate DNA synthesis and cell proliferation. And to explain the pathological changes of arteries caused by the antibodies, the effects of the antibodies on the proliferation of VSMCs were detected. Stimulation of VSMCs with the antibodies (1:40) resulted in an increase of DNA synthesis with time. The role was similar to that of NE, and could be blocked by α_1-adrenoceptor antagonist prazosin (Figure 3A), while IgG from normal Wistar rats had no effects. The effects of the antibodies on VSMC proliferation increased with the enhancement of the concentrations (Figure 3B). To confirm this result, the proliferative index was set up by flow

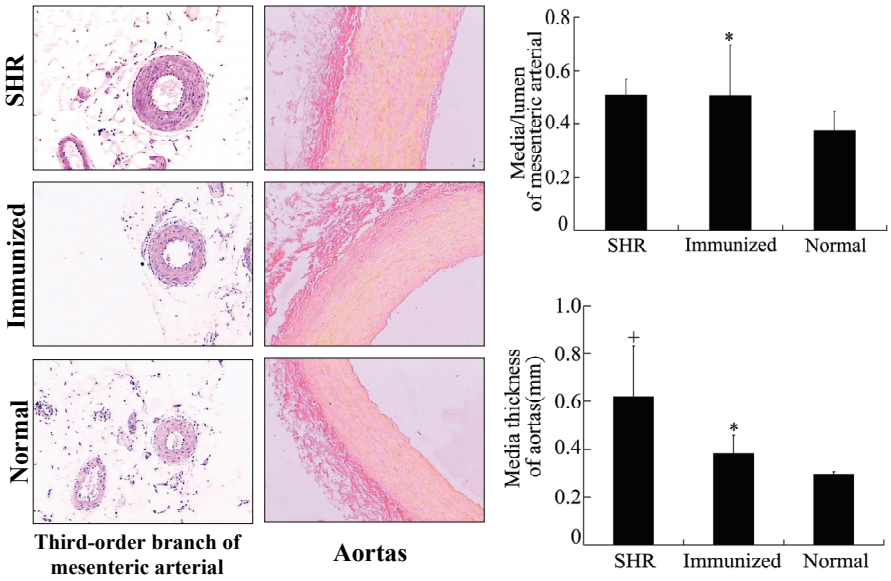

Figure 1. The pathological changes of arteries of the immunized rats. Cross sections of the third-order branch of the mesenteric arteries (hematoxylin-eosin) and aortas (van Gieson) were stained as described in Materials and Methods. *$p < 0.05$ *vs* normal; $^{+}p < 0.01$ *vs* immunized rats. Magnification: ×100.

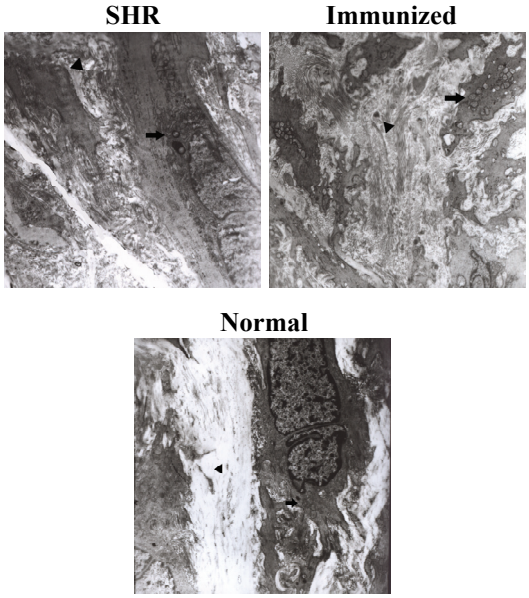

Figure 2. **Ultrastructural changes of aorta.** The ascending aorta was rinsed, fixed and observed under transmission electron microscope (×5,000 magnification). Two rats per group were analyzed and representative fields were shown. ➔Mitochondria; ▲ collagen fibril.

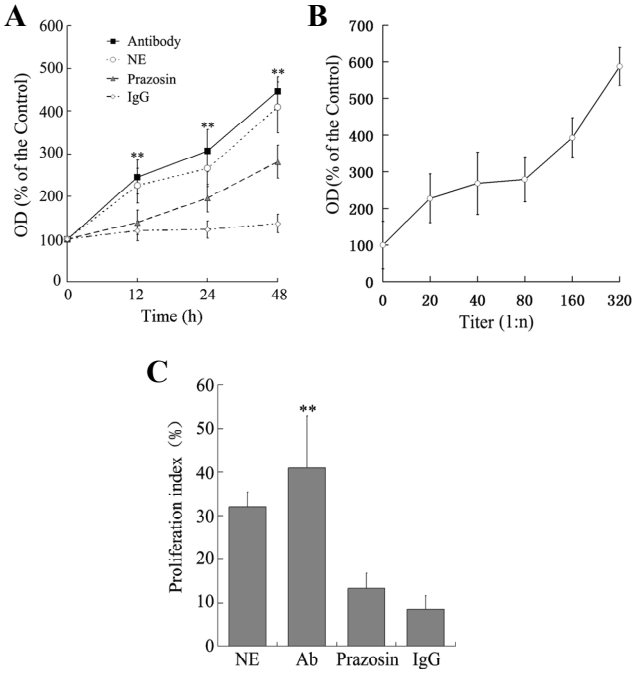

Figure 3. Induction of the proliferation of cultured rat VSMCs by antibodies against the α_1-adrenoceptor. (A) The time course of DNA synthesis of VSMCs detected by BrdU assay (n = 6 for each group and each time point). (B) The change of DNA synthesis of VSMCs with the titers of the antibodies after 24 h-stimulation (n = 6 for each titer). (C) The proliferative index of VSMCs measured by flow cytometry (n = 6-10 for each group). $^{**}p < 0.01$, compared with IgG control and prazosin group.

cytometry. The proliferation index caused by the antibodies was significantly higher than that of IgG control, and could be antagonized by prazosin (Figure 3C).

Antibodies against α_1-adrenoceptor increased the expression of c-jun in cultured VSMCs

c-fos, c-jun and c-myc oncogenes were the important factors to promote cell proliferation. Previous studies indicated α_1-adrenoceptor stimulation increased the expressions of these genes in aortas, VSMCs and other tissues or cells (21). In this paper, we studied the effects of antibodies against α_1-adrenoceptor on the expressions of c-jun and c-fos in cultured VSMCs. Exposure to the antibodies against α_1-adrenoceptor (1:40) caused the increase of c-jun mRNA expression, similar with the result that exposure to NE (100 μmol/L), while control IgG could not. Prazosin could block the role of the antibodies (Figure 4A). Although NE could promote the mRNA expression of c-fos, the antibodies against α_1-adrenoceptor had no such effect (Figure 4A). The protein expressions of c-jun (Figure 4B) and c-fos (Figure 4C) detected by Western blotting were resembled with their mRNA expressions.

To confirm the effects of the antibodies against α_1-adrenoceptor on the expressions of c-jun and c-fos, immunohistochemistry was performed. In the aortas of immunized rats, immunostaining for c-jun was intensely positive in VSMCs, while VSMCs from normal control exhibited

Table 3. The vascular morphological analyses

	SHR (n = 4)	Immunized (n = 9)	Normal (n = 10)
Outer diameters of mesenteric arteries (μm)	$57.2 \pm 2.7^{\Delta\Delta}$	42.9 ± 3.8	43.7 ± 7.9
Lumen diameters of mesenteric arteries (μm)	28.8 ± 2.1	21.7 ± 4.7	25.0 ± 4.7
Media width (μm)	$14.2 \pm 0.3^{\Delta\Delta}$	10.6 ± 2.5	9.4 ± 1.1
Media/lumen of mesenteric arteries	$0.50 \pm 0.03^{\Delta\Delta}$	0.53 ± 0.21*	0.38 ± 0.07
Media cross-section area of mesenteric arteries (μm^2)	$1920.8 \pm 147.8^{\Delta\Delta}$	1187.6 ± 182.5**	939.5 ± 116.1
Media of aortas (mm)	$0.62 \pm 0.21^{\Delta\Delta}$	0.38 ± 0.08* (n = 6)	0.30 ± 0.01 (n = 5)

$^{*}p < 0.05$, *vs* normal; $^{**}p < 0.01$, *vs* normal; $^{\Delta\Delta}p < 0.01$, *vs* immunized rats.

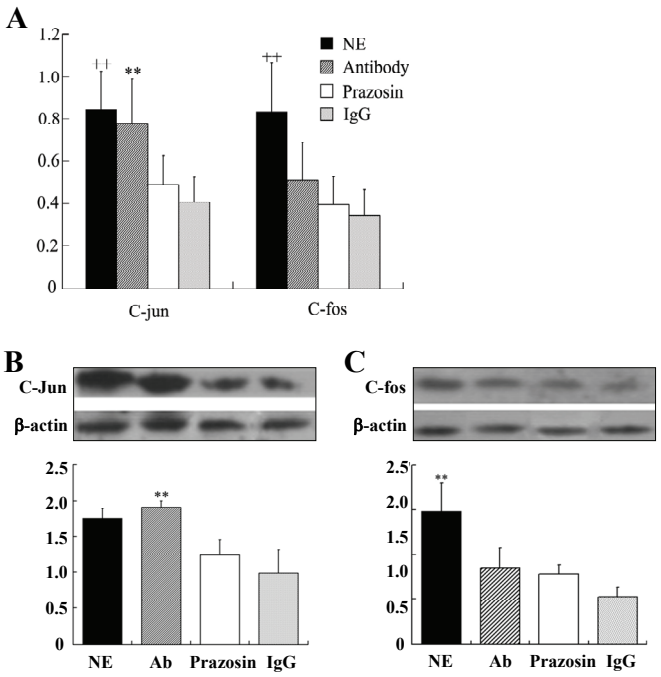

Figure 4. The expression of c-jun and c-fos in cultured VSMCs. (A) Detection of the expressions of c-jun and c-fos at the mRNA level by RT-PCR (n = 6). (B) The protein expression of c-jun by Western blotting (n = 4). (C) The protein expression of c-fos by Western blotting. $^{**}p < 0.01$ *vs* prazosin group and control IgG group. $^{++}p < 0.01$ *vs* other groups (n = 4).

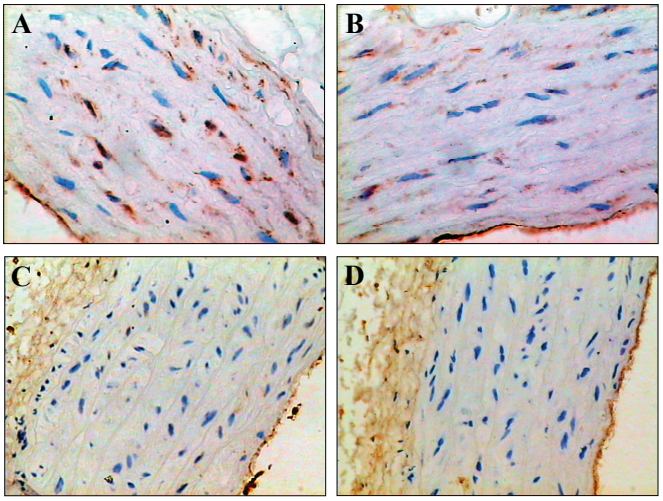

Figure 5. Detection of c-jun and c-fos in VSMCs by immunohistochemistry. (A) c-jun expression in immunized rats; (B) c-jun expression in normal control; (C) c-fos expression in immunized rats; (D) c-fos expression in normal control. The positive cells were in brown (magnification: A and B, ×400; C and D, ×200).

markedly reduced levels of staining. Instead, very low level of c-fos expression was showed in the VSMCs of the immunized rats and normal control (Figure 5).

The mRNA expression of α_1-adrenoceptor in cultured VSMCs

Chronic stimulation by catecholamines resulted in the downregulation of α_1-adrenoceptor in several tissues, including vascular smooth muscle (22, 23). To evaluate the change of α_1-adrenoceptor expression caused by the antibodies against α_1-adrenoceptor in VSMCs, α_1-adrenoceptor expression was quantified by RT-PCR. The results showed that in comparison with the control, no difference in the mRNA expressions of the α_{1A}-adrenergic receptors and α_{1B}-adrenergic receptors was found in the antibody group and control, and their expressions had no significant difference at the time of 0 h, 24 h, and 48 h (Figure 6A). But the mRNA level of α_{1D}-adrenoceptor in the antibody group was significantly lower than that of the control, and decreased gradually with time (Figures 6A and 6B).

Discussion

This was the first study on which chronic antibodies against α_1-adrenoceptor stimulation had effects the vascular damages of rats by exciting α_1-adrenoceptor *in vivo*. The study presented here demonstrated the antibodies against α_1-adrenoceptor with agonist-like activity could cause vascular remodeling including VSMC proliferation and the interstitial collagen deposition. The antibodies were likely due to the transmission of the exciting effect of α_1-adrenergic agonists by the α_{1A}-adrenergic receptor subtype in our system, because the data showed the preference of prazosin could antagonized the antibody-induced effect on the proliferation of VSMCs.

Remodeling of the resistance vasculature played a key role in the pathogenesis of essential hypertension and was an important reason of refractory hypertension. In hypertension, resistance arteries undergo eutrophic and/or hypertrophic remodeling. In inward eutrophic remodeling, outer and lumen diameters are reduced, media cross-section area is unaltered, and media/lumen ratio is increased. Eutrophic remodeling allows the vessels to maintain an increased resistance in essential hypertension (24). This study found that chronic stimulation in rat's arteries with antibodies against the α_1-adrenoceptor increased media-lumen ratio of small resistance arteries, increased media area and collagen content of big arteries, and the changes mainly exhibited eutrophic remodeling. This result explained our early findings that the frequencies of autoantibodies against the α_1-adrenoceptor were higher particularly in the patients with refractory hypertension (6).

The growth of VSMCs is the mechanism that is more classically associated with vascular remodeling, although autoantibodies against the α_1-adrenoceptor have agonist activity like NE, and α_1-adrenoceptor stimulation has affirmative role in VSMCs (10, 11). The roles of the antibodies on VSMCs are completely unclear before. To further confirm the changes of vascular remodeling induced by the antibodies, the proliferation of cultured VSMCs

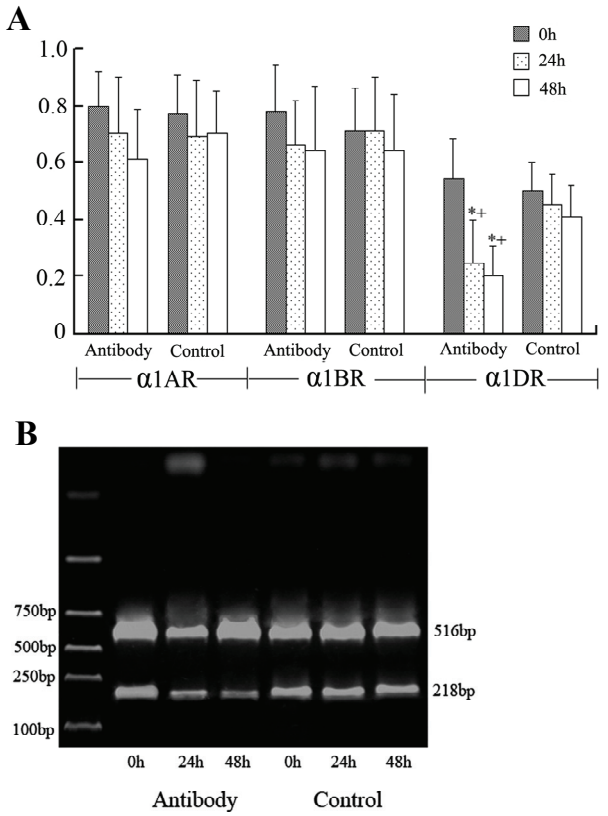

Figure 6. The mRNA expression of $\alpha_{1A,B,D}$-adrenoceptors by RT-PCR. (A) The densities of $\alpha_{1A,B,D}$-adrenergic receptor bands relative to GAPDH. $^{*}p < 0.05$ *vs* 0 h, $^{+}p < 0.05$ *vs* control at the indicated time, n = 6. (B) The expression of α_{1D}-adrenoceptor.

induced by the antibodies was measured. Our data suggested the artery remodeling can be formed by the VSMC proliferation induced by antibodies against α_1-adrenoceptor, and may be antagonized by prazosin. In this paper, the collagen synthesis *in vitro* was not detected in VSMCs, but the pathological changes, especially ultrastructural changes of arteries, certified that the antibodies could increase the collagen synthesis which was a characteristic of vascular remodeling in hypertension.

Previous study showed that in the mechanisms of VSMC proliferation induced by NE, in this procedure, the transcription factors such as c-fos, c-jun, and c-myc exhibited critical contributions (25). By measuring these transcription factors, we found the antibodies could increase the expression of c-jun in VSMCs, and no difference of c-fos expression was found. But c-myc was not completely expressed in the antibodies-treated VSMCs and the control *in vivo* and *in vitro* (data not shown). The mechanisms of the differences on the transcription factor expressions between antibodies against the α_1-adrenoceptor and NE were not clear. The causes may be: the antibodies could induce chronotropic effect while NE could not (3-5), or NE could stimulate other kinds of receptors.

In this study, the facts that the blood pressure had no elevation in immunized group suggested the autoantibody may not be a factor to cause high blood pressure, but an agent to promote pathological changes in hypertension. The apparent discrepancies between resistance vessel structure and blood pressure in immunized rats are likely due to the fact that blood pressure is not only dependent on vascular structure but also on the flow through them and the hormonal environment (26). In this paper, although the media-lumen ratio in immunized group was similar to that of the SHR, the HR were significantly lower than that of the SHR. So the cardiac outputs may be significantly reduced, and this may be a reason that the blood pressure did not rise. On the other hand, the expression of the α_{1D}-adrenoceptor on VSMCs was decreased in antibody group, which was the same with our earlier study. In that research, the mRNA expression of the α_{1D}-adrenergic receptor in the heart of immunized rats was significantly lower than that of the normal. Because the α_{1D}-adrenoceptor participated directly in sympathetic regulation of systemic blood pressure by vasoconstriction (27, 28), the reduced expression of α_{1D}-adrenoceptor may be one of the reasons which made the blood pressure not rise in the immunized rats.

In summary, antibodies against the α_1-adrenoceptor stimulated the growth of VSMCs caused by increased expression of c-jun, and may contribute to vascular damages. To those hypertensive patients with the autoantibodies, the therapy using α_1-adrenoceptor antagonist to block the autoantibodies is necessary, because in the condition with the autoantibodies against the α_1-adrenoceptor, the pathomorphology may be progressing even if the blood pressure is maintained at normal level by other drugs.

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