

Increase in G protein-coupled receptor autoantibodies with decline of cardiac function in hypercholesterolemic rats

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Abstract. – OBJECTIVE: This study aimed to explore the effects of diet-induced hypercholesterolemia (HC) on the production of G protein-coupled receptor autoantibodies and to elucidate the potential mechanisms involved.

MATERIALS AND METHODS: Male Wistar rats were fed a normal or high-cholesterol diet for 8 weeks. Cardiac function, autoantibodies against G protein-coupled receptors, the beat frequency of neonatal cardiomyocytes, the CD4+/CD8+ T-lymphocyte ratio and lymph leukocyte counts in the spleen were determined.

RESULTS: Diet-induced hypercholesterolemia significantly increased the levels of autoantibodies against α 1- and β 1-adrenergic receptors and the angiotensin II type 1 receptor in sera, as well as the CD4+/CD8+ T-lymphocyte ratio and lymph leukocyte count in the spleen, and decreased cardiac function. There were strong negative correlations between the levels of autoantibodies and cardiac injury.

CONCLUSIONS: The present study demonstrates, for the first time, that G protein-coupled receptor autoantibodies exist in the sera of hypercholesterolemic rats and that the levels of these autoantibodies are related to cardiac function, which implies that these cardiac receptor autoantibodies may play a role in cardiac dysfunction in hypercholesterolemic rats.

Key Words:

Hypercholesterolemia, Autoantibody, G protein-coupled receptor, Cardiac function.

Introduction

Hypercholesterolemia is one of the most important risk factors for atherosclerosis and cardiovascular diseases. According to the clinical aetiology and pathogenesis of hypercholesterolemia, dietary control, exercise therapy, lipid-lowering medication, lipid separation, liver transplantation, and gene therapy are among the main measures used to clinically regulate lipid disorders¹⁻³. However, none of these measures are completely effective. Therefore, it is necessary to identify new therapeutic targets or more effective control measures to maintain stable blood lipid levels or reduce the occurrence of lipid disorders.

Several studies have demonstrated that hypercholesterolemia can induce changes in cardiac structure that lead to further myocardial remodelling and that these changes may be due to the oxidative stress induced by high fat^{4,5}, activation of the adrenaline and angiotensin systems⁶, and increases in endothelin-1 expression. Myocardial remodelling can lead to exposure to autoantigens that are normally concealed. Exposure to autoantigens may be one of the reasons for the production of autoantibodies⁷. Moreover, a recent study found that the immune system response participates in hypercholesterolemia and subsequent ath-

erosclerosis, which includes increases in the serum complement system, changes in serum cytokines and the formation of circulating immune complexes in hypercholesterolemic patients¹⁰⁻¹⁴. Since the 1990s, serum autoantibodies against G protein-coupled receptors, such as autoantibodies against α_1 -adrenergic receptors (α_1 -AA), β_1 -adrenergic receptors (β_1 -AA), β_3 -adrenergic receptors (β_3 -AA), M_2 -cholinergic receptors (M_2 -AA) and angiotensin-II type 1 receptors (AT1-AA), have been detected in patients with dilated cardiomyopathy, rheumatic heart disease, Chagas disease, hypertension and primary electrocardiographic disorders¹⁵⁻¹⁹. These findings further suggest that hypercholesterolemia may be related to autoantibodies. Some research has demonstrated that the autoimmune response may play a role in the development of heart disease and heart failure induced by a variety of factors. Autoantibodies against G protein-coupled receptors can not only block the specific site of the receptor but can also exert agonist-like effects on specific receptors and their downstream signalling pathways, altering their normal regulatory functions. Cardiac structure and function are consequently further deteriorated. These results suggest that G protein-coupled receptor autoantibodies may play an important role in the development of cardiovascular diseases. Evidence has demonstrated that hypercholesterolemia causes ultrastructural changes in the myocardium and inflammatory responses in cardiovascular diseases as well as immune system dysfunction. These lines of evidence indicate that hypercholesterolemia is likely to induce immune system dysfunction and subsequent production of G protein-coupled receptor autoantibodies. If this chain of events is accurate, these autoantibodies may increase the vulnerability of the myocardium in hypercholesterolemic conditions and aggravate heart injury. Hence, exploration of whether hypercholesterolemia induces the production of G protein-coupled receptor autoantibodies should not be ignored in studies of cardiovascular diseases.

Therefore, the present study sought to achieve the following goals: (1) to assess whether autoantibodies against G protein-coupled receptors can be induced by high cholesterol diet-induced hypercholesterolemia and (2) to observe and analyze the relationship between the emergence of autoantibodies and cardiac function in hypercholesterolemic rats.

Materials and Methods

Animals and Lipid Detection

Healthy male Wistar rats weighing 110 ± 10 g that were serum α_1 -AA-, β_1 -AA-, β_3 -AA-, M_2 -AA- and AT1-AA-negative were provided with food and water ad libitum. Blood was drawn by cutting the tail of each rat, and plasma lipid baseline values were determined with assay kits (Cholesterol Kit, Triglycerides Kit, and Direct LDL-Cholesterol Kit, BioSino Bio-technology and Science Inc., China). Next, the rats were randomly divided into two groups: the control group ($n=18$) was given a normal diet, and the high-cholesterol group (HC group, $n=18$) was given a high-cholesterol diet (normal diet supplemented with 1% cholesterol, 10% egg yolk and 5% lard) for 8 weeks. Four and eight weeks after the initiation of the high-cholesterol diet, the rats' blood was redrawn, and their plasma lipids were determined. The experiments were performed in accordance with the National Institutes of Health Guidelines on the Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals protocol published by the Ministry of the People's Republic of China (issued on June 3, 2004) and were approved by the Institutional Committee on Animal Care of Shanxi Medical University.

Determination of Myocardial Function

At the end of 8 weeks, the rats were anesthetized with 2% sodium pentobarbital (Sigma-Aldrich, St. Louis, MO, USA) (50 mg/kg) via peritoneal injection. Cardiac function was assessed based on the left ventricular pressure (LVP)²⁰. LVP was measured using a polyethylene catheter inserted into the left ventricular cavity through the left carotid artery and was digitally processed with a hemodynamic analysis system (MS2000 biology signal analysis system, TECHMAN SOFT, Chengdu, China). The left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), and maximal positive and negative values of the instantaneous first derivative of the LVP ($+dP/dt_{\max}$ and $-dP/dt_{\max}$) were derived with computer algorithms. After recording, the spleen was immediately excised and processed according to the procedures described below for fluorescence-activated cell sorting (FACS) analysis.

Measurement of Autoantibodies Against G Protein-coupled Receptors

After 0 and 4 weeks and at the end of 8 weeks, 1-ml blood samples were collected by cutting the tail of each rat. The sera were then separated and stored at -70°C for the following analyses.

Five types of peptides corresponding to the sequences of the second extracellular loops of the rat G protein-coupled receptors (α_1 -adrenergic receptor: G-W-K-E-P-V-P-P-D-E-R-F-C-G-I-T-E-E-A-G-Y-A-V-F-S-S-V; β_1 -adrenergic receptor: H-W-W-R-A-E-S-D-E-A-R-R-C-Y-N-D-P-K-C-C-D-F-V-T-N-R-C; β_3 -adrenergic receptor: R-V-G-A-D-A-E-A-Q-E-C-H-S-N-P-R-C-C-S-F-A-S-N-M-P; M_2 -cholinergic receptor: V-R-T-V-E-D-G-E-C-Y-I-Q-F-F-S-N-A-A-V-F-G-T-A-I-C; and angiotensin-II type 1 receptor: A-V-I-H-R-N-V-Y-F-I-E-N-T-N-I-T-V-C-A-F-H-Y-E-S-R-N-S-T-L-P) were commercially synthesized by the Institute of Biochemistry and Cell Biology (Shanghai Institute for Biological Sciences, Chinese Academy of Sciences). The purity of 95% was ensured for the peptides via high-performance liquid chromatography (HPLC) analysis. The peptides were stored in 0.1 M Na_2CO_3 at -20°C .

Autoantibodies against the G protein-coupled receptors were detected with an enzyme-linked immunosorbent assay (ELISA) method that has been described previously²¹, and the samples were analyzed in duplicate. The wells were then saturated with PBS supplemented with 3% (w/v) skimmed milk, 0.1% (v/v) Tween-20 (Sigma-Aldrich, St. Louis, MO, USA) and 0.01% (w/v) thimerosal (Sigma-Aldrich, St. Louis, MO, USA) (PMT), followed by incubation for 1 h at 37°C . After washing the wells three times with PMT, 50 μl of rat serum (1:20 dilutions in PMT) was added to the saturated microtiter plates, followed by incubation for 2 h at 37°C . After washing three times with PMT again, an affinity-purified biotinylated rabbit anti-rat IgG (H+L) antibody (Jackson Immunoresearch Laboratories, Inc., PA, USA; 1:2000 dilution in PMT) was allowed to react for 1 h at 37°C . After three additional washes, the bound antibody was detected by incubating the plates for 1 h with a streptavidin-peroxidase (1 $\mu\text{g}/\text{ml}$, Sigma-Aldrich, St. Louis, MO, USA) solution in PMT. Three washes in PBS and the addition of 50 μl of 2.5 mM H_2O_2 and 2 mM 2,2'-azino-di (3-ethyl-benzthiazoline-6-sulfonic acid, ABTS; Sigma-Aldrich, St. Louis, MO, USA) were then performed, followed by incubation for 30 min at 37°C . Next, the optical density (OD) values were read at 405 nm in a microplate reader (SpectraM2e, Molecular Devices, Sunnyvale, CA, USA).

Purification of Immunoglobulin G from Rat Sera

At the end of cardiac function analysis and recording, blood was drawn from the abdominal

aorta of each rat. Based on the results of ELISA detection, α_1 -AA-, β_1 -AA- and AT1-AA-positive rats and α_1 -AA-, β_1 -AA- and AT1-AA-negative rats were chosen. Immunoglobulin G fractions (IgG) were purified from rat serum samples with the Mab Trap Kit (GE Healthcare, Chicago, IL, USA), according to previously reported methods²². The concentrations (mg/ml) and specificities of the purified IgGs were determined through the Bicinchoninic Acid (BCA) Protein Assay (Pierce, Appleton, WI, USA) and ELISA, respectively.

Culture of Beating Neonatal Cardiomyocytes

The hearts of 1-to-3-day-old Wistar rats were removed aseptically and cultured as described previously²³. The number of beats of a selected isolated myocardial cell or a cluster of synchronously contracting cells was counted for 15 s in each of 10 fields. The IgG fractions from α_1 -AA-, β_1 -AA- and AT1-AA-positive rats and the corresponding receptor antagonists (the α_1 -adrenergic receptor antagonist prazosin (Sigma-Aldrich, St. Louis, MO, USA), the β_1 -adrenergic receptor antagonist metoprolol (Sigma-Aldrich, St. Louis, MO, USA) and the angiotensin-II type 1 receptor antagonist valsartan (Sigma-Aldrich, St. Louis, MO, USA) were then added, and the cells were observed for 5 min after each addition. This procedure was repeated twice in different cultures to yield results representing a total of 30 cells or cell clusters. The basal beating rate was 80 ± 15 beats/min.

Detection of the CD4⁺/CD8⁺ T-lymphocyte Ratio and Lymph leukocyte Counts in the Spleen through FACS Analysis

Single spleen cell suspensions were prepared by grinding rat spleens between two frosted glass slides in a medium consisting of RPMI 1640 with 25 mM HEPES and 0.5% BSA. The suspensions were then homogenized in Ficoll lymphocyte extraction buffer, and the soluble extracts were centrifuged at 2500 rpm for 30 min to obtain the cells of the mononuclear cell layer. These cells were then washed twice with medium (incomplete RPMI 1640 culture medium without BSA). Next, the peripheral blood mononuclear cells (PBMCs) and RBC-free splenocytes were suspended in FACS buffer (PBS containing 2% v/v FBS and 0.1% NaN_3 , EB) to produce a concentration of 10,000 to 20,000 cells/ml. All of the cell preparations obtained were 90% viable, as determined by trypan blue exclusion.

For the immunofluorescence analysis, the cells (1×10^6 cells) were stained for 30 min at 4°C with optimal concentrations of PE-conjugated anti-rat CD4 and FITC-conjugated anti-rat CD8 (Biolegend, San Diego, CA, USA) or PE-conjugated anti-rat CD45R (eBioscience Laboratories, San Diego, CA, USA) antibodies. Staining for the IgG2b isotype was performed using a PE-conjugated monoclonal mouse IgG2b antibody (eBioscience Laboratories, San Diego, CA, USA). Following washes, the cells were placed on ice and immediately acquired on a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA, USA), running CellQuest software. The forward and light angle scatter gates were set to exclude non-lymphoid cells. The immune status was evaluated based on CD4+/CD8+ cells, and the lymphocyte content was detected with CD45R.

Statistical Analysis

The data are expressed as the mean \pm SEM. Comparisons between groups were performed with 2-sample Student's *t*-tests. Repeated measures analyses were conducted with general linear models. Linear correlation analysis was carried out to evaluate the emergence of autoantibodies and cardiac function. All statistical tests were two-sided, and reported *p*-values were considered significant if less than 0.05. All analyses were performed using SPSS 16.0 (IBM SPSS, Inc., Chicago, IL, USA).

Results

High-cholesterol Diet-induced Hypercholesterolemia

In addition to genetic factors, hypercholesterolemia is strongly induced by an improper diet or excessive

and long-term intake of high-calorie, high-cholesterol, and high-fatty acid diets. In the present study, there were no significant differences between the groups in terms of the plasma lipid profiles before the onset of the study (time 0). Eight weeks of feeding on the high-cholesterol diet resulted in dramatic increases in plasma cholesterol (CHO, 3.46 ± 0.68 mmol/L vs. 1.28 ± 0.22 mmol/L, $p < 0.01$), triglycerides (TG, 1.17 ± 0.39 mmol/L vs. 0.26 ± 0.08 mmol/L, $p < 0.01$) and low-density lipoprotein cholesterol (LDL-Cho, 1.67 ± 0.35 mmol/L vs. 0.48 ± 0.09 mmol/L, $p < 0.01$) (Table I). These results indicated that the high-cholesterol diet-induced increases in plasma lipid levels; thus, the diet-induced hypercholesterolemic rat model was successfully established.

Hypercholesterolemia Worsens Cardiac Functional and Induces Increases in Serum α_1 -AA, β_1 -AA and AT1-AA

The high-cholesterol diet markedly degraded myocardial contractile function. Compared with the normal diet group, the LVSP, $+dP/dt_{\max}$ and $-dP/dt_{\max}$ values were significantly decreased in the high-cholesterol diet group (LVSP: 13.64 ± 1.37 kPa vs. 20.79 ± 2.87 kPa, $p < 0.01$; $+dP/dt_{\max}$: 537.43 ± 102.24 kPa/s vs. 953.38 ± 171.12 kPa/s, $p < 0.01$; and $-dP/dt_{\max}$: 452.19 ± 88.34 kPa/s vs. 793.10 ± 112.82 kPa/s, $p < 0.01$; Figure 1).

Compared with the normal diet group, α_1 -AA, β_1 -AA and AT1-AA levels had significantly increased in the high-cholesterol diet group by the 4th week (α_1 -AA: 0.60 ± 0.05 vs. 0.27 ± 0.06 , $p < 0.01$; β_1 -AA: 0.57 ± 0.06 vs. 0.18 ± 0.02 , $p < 0.01$; and AT1-AA: 0.53 ± 0.067581 vs. 0.16 ± 0.04 , $p < 0.01$). After 4 additional weeks of the high-cholesterol diet, these antibodies exhibited slight downward trends in the serum (Figure 2). However, no significant differences in β_3 -AA or M₂-AA levels were observed between

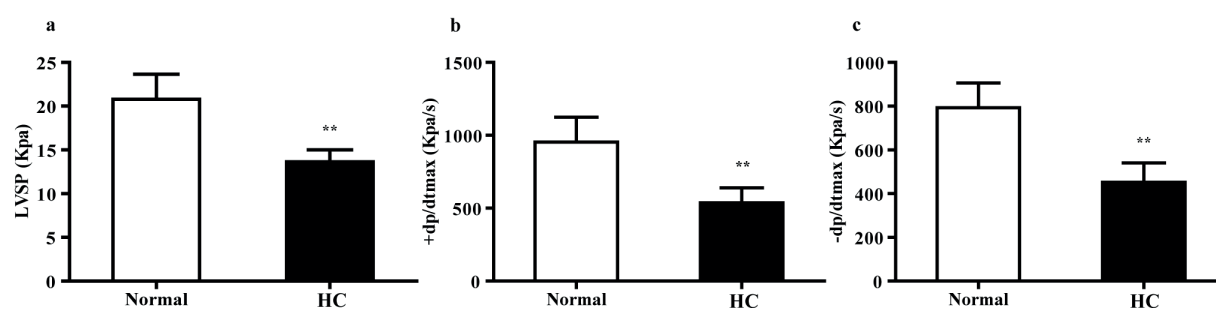


Figure 1. Baseline LVSP (a), left ventricular $+dP/dt_{\max}$ (b), and left ventricular $-dP/dt_{\max}$ (c) in the normal and high-cholesterol diet groups. Normal, normal diet group; HC, high-cholesterol diet group. The same abbreviations are used in all figures. ** $p < 0.01$ vs. normal. $N=15/\text{group}$.

Table 1. Lipid profiles in normal diet and high-cholesterol diet groups.

	CHO (mmol/L)		TG (mmol/L)		LDL (mmol/L)	
	Normal	HC	Normal	HC	Normal	HC
0 weeks	1.27±0.23	1.28±0.22	0.25±0.04	0.26±0.08	0.46±0.04	0.48±0.09
4 weeks	1.28±0.27	2.69±0.27	0.26±0.05	0.88±0.35	0.47±0.10	1.00±0.21
10 weeks	1.43±0.16	3.46±0.68	0.29±0.05	1.17±0.39	0.52±0.07	1.67±0.35

Values are expressed as the mean±SEM, n = 18 rats per group. ND, normal diet; HC, high-cholesterol diet; CHO, cholesterol; TG, triglyceride; LDL, low-density lipoprotein.

the normal diet and the high-cholesterol diet groups. These findings indicated that hypercholesterolemia likely induced G protein-coupled receptor autoantibody production, and these autoantibodies may have been closely related to the cardiac dysfunction in the hypercholesterolemic rats.

α_1 -AA-, β_1 -AA- and AT1-AA-Positive IgGs Increase the Beat Frequency of Cultured Cardiomyocytes

Spontaneously beating rat neonatal cardiomyocytes were used to study the functional effects of the antibodies. The IgG fractions (10^{-6} mol/L) isolated from the α_1 -AA-, β_1 -AA- and AT1-AA-positive sera of rats increased the cardiomyocyte beat frequency. These effects of α_1 -AA, β_1 -AA, and AT1-AA were abolished by the addition of the α_1 -adrenergic receptor antagonist prazosin (10^{-6} mol/L), the β_1 -adrenergic receptor antagonist metoprolol (10^{-6} mol/L) and the angiotensin-II type 1 receptor antagonist valsartan (10^{-5} mol/L) (Figure 3).

These data reveal that IgGs from α_1 -AA-, β_1 -AA- and AT1-AA-positive sera can increase the beat frequency of newborn rat cardiomyocytes by activating the α_1 -adrenergic receptors (α_1 -AR), β_1 -adrenergic receptors (β_1 -AR), angiotensin-II type 1 receptors (AT1-AR).

The Correlations Between the Changes in Myocardial Contractile Function Induced by Hypercholesterolemia and Autoantibody Levels in the Serum are Negative

To determine the direct relationships between cardiac function and autoantibody levels, the correlations of +dP/dt_{max} (an indicator of cardiac function) with serum G protein-coupled receptor autoantibody levels were analyzed. Here, +dP/dt_{max} was negatively correlated with α_1 -AA, β_1 -AA and AT1-AA ($r = -0.627$, $p < 0.01$; $r = -0.532$, $p < 0.01$; and $r = -0.575$, $p < 0.01$, respectively; Figure 4).

These results suggest that hypercholesterolemia-induced cardiac impairment may be associated with the production of some G protein-coupled receptor autoantibodies.

The Spleen CD4⁺/CD8⁺ T-lymphocyte Ratio and Lymph Leukocyte Counts are Increased in High-cholesterol Diet-fed Rats

To further elucidate the production of G protein-coupled receptor autoantibodies in hypercholesterolemia, immune system functions were examined based on the spleen CD4⁺/CD8⁺ T-lymphocyte ratio and lymph leukocyte counts.

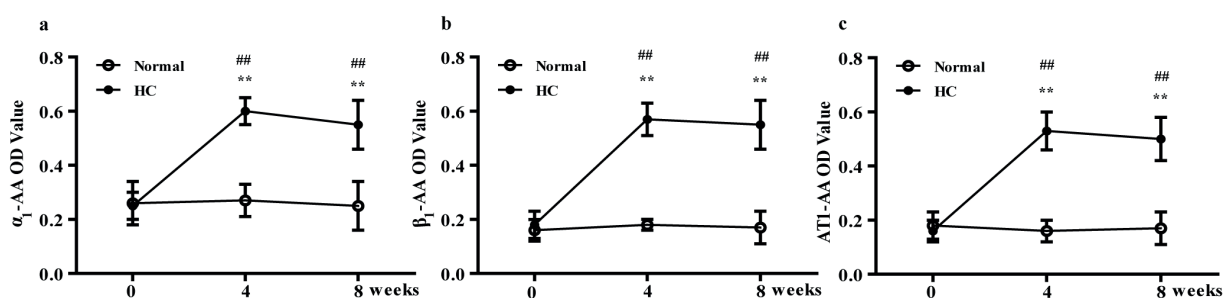


Figure 2. Hypercholesterolaemia increases the OD values of some cardiac autoantibodies against G protein-coupled receptors in the sera of rats. (a) OD value of α_1 -AA, (b) OD value of β_1 -AA, and (c) OD value of AT1-AA. ** $p < 0.01$ vs. 0 weeks, ### $p < 0.01$ vs. normal. N=18/group.

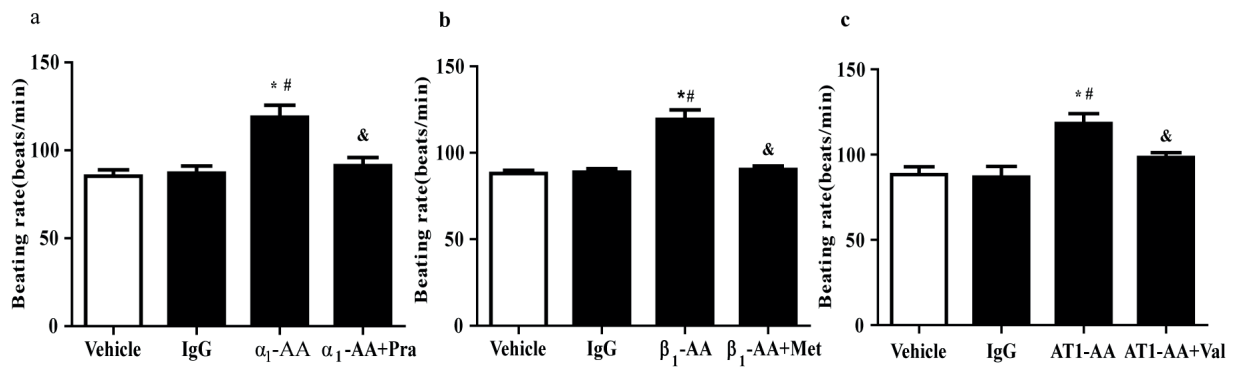


Figure 3. Increased beating rate of isolated myocardial cells stimulated by the IgG fractions from α_1 -AA-, β_1 -AA- and AT1-AA-positive rat sera (a, α_1 -AA; b, β_1 -AA; c, AT1-AA). Vehicle, saline; IgG, negative immunoglobulin G fractions; α_1 -AA, immunoglobulin G fractions from α_1 -AA-positive rat sera; Pra, Prazosin; β_1 -AA, immunoglobulin G fractions from β_1 -AA-positive rat sera; Met, Metoprolol; AT1-AA, immunoglobulin G fractions from AT1-AA-positive rat sera; Val, Valsartan. ^{*} $p < 0.05$ vs. vehicle, [#] $p < 0.05$ vs. IgG; [&] $p < 0.05$ vs. the α_1 -AA, β_1 -AA and AT1-AA groups. N=6/group.

FACS analysis demonstrated that compared with the normal diet group, in the high-cholesterol diet group, the CD4⁺/CD8⁺ ratio (2.34 ± 0.16 vs. 1.61 ± 0.21 , $p < 0.05$) and the content of lymph leukocytes (DC45R⁺ cells, 25.34 ± 3.18 vs. 7.64 ± 3.47 , $p < 0.01$) were increased significantly (Figure 5). These results demonstrated that hypercholesterolemia induced changes in the splenic immune system and further suggest that elevation of blood lipid levels and subsequent changes may affect immunologic functions and induce immune disorders, resulting in the genesis of G protein-coupled receptor autoantibodies.

Discussion

Our findings confirmed that a high-cholesterol diet induced a plasma lipid disorder and caused

cardiac dysfunction. Through SA-ELISA screening, the current study was the first to discover the existence of autoantibodies against α_1 -adrenergic receptors, β_1 -adrenergic receptors and angiotensin-II type 1 receptors in the sera of hypercholesterolemic rats, which were present at levels that were significantly higher than those in rats fed a normal diet. Furthermore, the autoantibodies exhibited functions that increased the beat frequency of newborn rat cardiomyocytes. Moreover, strong negative correlations were found between hypercholesterolemia-induced myocardial contractile dysfunction and serum autoantibody levels. These results suggested that hypercholesterolemia may induce the production of the serum autoantibodies mentioned above and that these autoantibodies may be closely related to cardiac dysfunction.

Since autoantibodies against the β_1 -adrenergic receptor were found in the sera of patients

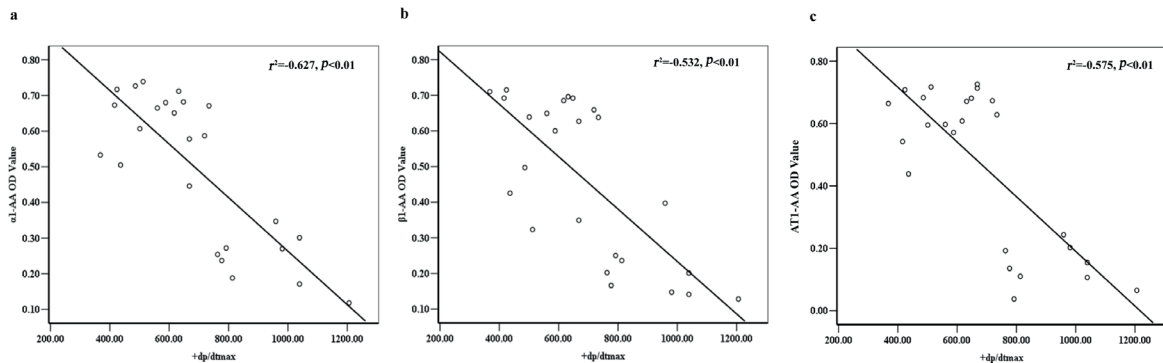


Figure 4. Correlation analyses of baseline $+dp/dt_{max}$ with the OD values for some autoantibodies against G protein-coupled receptors in the normal diet and high-cholesterol diet groups. There were significant negative correlations of the OD values of the autoantibodies with $+dp/dt_{max}$ (a, α_1 -AA: $r^2 = -0.627$, $p < 0.01$; b, β_1 -AA: $r^2 = -0.532$, $p < 0.01$; c, AT1-AA: $r^2 = -0.575$, $p < 0.01$). N=25.

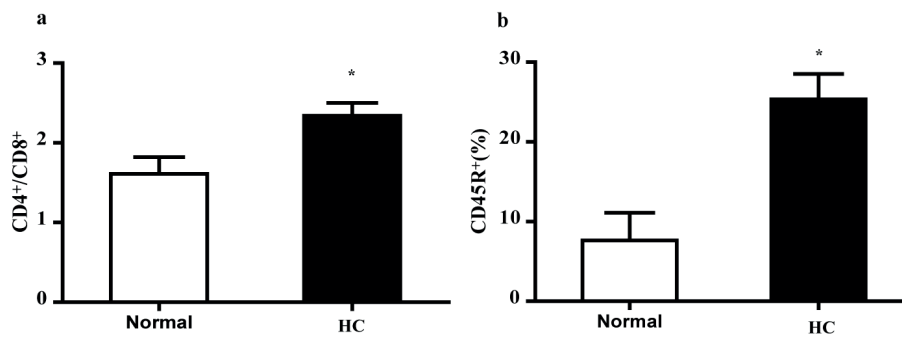


Figure 5. Hypercholesterolaemia induces immune system activation. (a) The spleen CD4⁺/CD8⁺ T-lymphocyte ratios and (b) lymph leukocyte contents of the spleens of the rats. * $p < 0.05$, ** $p < 0.01$ vs. normal. N=18/group.

with idiopathic dilated cardiomyopathy (IDCM) for the first time by Magnusson et al¹⁵ in 1990, researchers have detected many types of G protein-coupled receptor autoantibodies in the sera of patients with a variety of cardiovascular diseases and heart failure. Moreover, several G protein-coupled receptor autoantibodies can be simultaneously present in a single disease; for example, autoantibodies against cardiac β_1 -adrenergic receptors detected in the sera have been found to be prevalent in patients with IDCM²⁴. Moreover, AT1-AA can not only induce harmful changes in the structure and function of the heart but also cause hypertrophy of cultured neonatal rat cardiomyocytes²⁵. Studies have demonstrated that different degrees of cardiac remodelling, with different causes, may be among the causes of autoantibody production. Hypercholesterolemia may induce different extents of cardiac structural and myocardial remodelling through many mechanisms and subsequently lead to the production of autoantibodies against the α_1 -adrenergic receptor, the β_1 -adrenergic receptor and the angiotensin-II type 1 cholinergic receptor in the sera of rats. Several studies have demonstrated that multiple immune mechanisms may participate in the development of heart failure and cardiac remodelling and subsequently affect the occurrence of autoantibodies²³. The present study also found that spleen lymph leukocyte counts and the CD4⁺/CD8⁺ T-lymphocyte ratio were significantly increased in hypercholesterolemic rats, which suggest that hypercholesterolemia may activate the immune system and the ability to produce antibodies. These autoantibodies against G protein-coupled receptors might be related to alterations in the immune system.

The current experiments demonstrated that pathological changes in the heart may be attrib-

able to the production of some G protein-coupled receptor autoantibodies. A number of studies have reported that these anti-receptor antibodies can display functional activity in the development of diseases. For example, viral infection can lead to myocardial damage and induce autoantibody production. These autoantibodies can specifically bind to cognate receptors, exert agonist-like activities and thus affect cardiac structure and function and further worsen diseases. The majority of studies have demonstrated that the chronic stimulation of α_1 -adrenergic receptors by the agonist-like α_1 -AA can up-regulate *c-jun* expression, which promotes DNA synthesis and protein expression in cardiomyocytes and interstitial fibroblasts, in turn causing hypertrophy of cardiomyocytes and interstitial collagen deposition, ultimately leading to cardiac remodelling and an increase in peripheral resistance²⁶. β_1 -AA can increase the beating rate of cultured neonatal rat cardiomyocytes and increase myocardial oxygen consumption, and long-term stimulation by β_1 -AA can increase the cardiac load²⁷. Such long-term stimulation may also induce cardiomyocyte apoptosis and thus cause myocardial injury. Specific removal of β_1 -AA through immunoadsorption can significantly improve cardiac structure and function in experimental autoimmune cardiomyopathy²⁰. Roland Jahns et al²⁸ confirmed that long-term stimulation with β_1 -AA can induce IDCM. Perindopril treatment significantly decreases the frequency and geometric mean titer in patients positive for AT1-AA, even to complete ablation, and these patients show greater improvement in left ventricular remodelling and heart function²¹. Some studies have indicated that hypercholesterolemic patients produce autoantibodies and that the relationship between the autoimmune response to autoantibodies and

the extent of atherosclerosis is more complex²⁹. Taken together, our results suggest that cardiac G protein-coupled receptor autoantibodies may play an important role in the initiation and development of cardiovascular diseases³⁰. Furthermore, the present study demonstrated that the levels of α_1 -AA, β_1 -AA and AT1-AA are negatively correlated with cardiac function. The production and actions of these autoantibodies may be among the factors that cannot be ignored in hypercholesterolemia and the subsequent myocardial injury and cardiac dysfunction. However, the specific mechanisms and pathways involved remain to be confirmed.

Conclusions

We found for the first time that certain cardiac G protein-coupled receptor autoantibodies exist in the sera of hypercholesterolemic rats. Hypercholesterolemia-induced cardiac remodelling induces α_1 -AA, β_1 -AA and AT1-AA production and further affects cardiac function in rats. Blocking the production of cardiac G protein-coupled receptor autoantibodies during an earlier stage of hypercholesterolemia may attenuate cardiac injury and thus delay subsequent cardiovascular diseases. While we recognize that our findings are preliminary and require additional confirmation through a series of detailed mechanistic studies, we nonetheless suggest the possible role of immunological mechanisms in the pathogenesis of hypercholesterolemia.

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Conflict of interest

The authors declare no conflicts of interest.

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