

ORIGINAL RESEARCH ARTICLE

Role of IgE-FcεR1 in Pathological Cardiac Remodeling and Dysfunction

BACKGROUND: Immunoglobulin E (IgE) belongs to a class of immunoglobulins involved in immune response to specific allergens. However, the roles of IgE and IgE receptor (FcεR1) in pathological cardiac remodeling and heart failure are unknown.

METHODS: Serum IgE levels and cardiac FcεR1 expression were assessed in diseased hearts from human and mouse. The role of FcεR1 signaling in pathological cardiac remodeling was explored in vivo by FcεR1 genetic depletion, anti-IgE antibodies, and bone marrow transplantation. The roles of the IgE-FcεR1 pathway were further evaluated in vitro in primary cultured rat cardiomyocytes and cardiac fibroblasts (CFs). RNA sequencing and bioinformatic analyses were used to identify biochemical changes and signaling pathways that are regulated by IgE/FcεR1.

RESULTS: Serum IgE levels were significantly elevated in patients with heart failure as well as in 2 mouse cardiac disease models induced by chronic pressure overload via transverse aortic constriction and chronic angiotensin II infusion. Interestingly, FcεR1 expression levels were also significantly upregulated in failing hearts from human and mouse. Blockade of the IgE-FcεR1 pathway by FcεR1 knockout alleviated transverse aortic constriction- or angiotensin II-induced pathological cardiac remodeling or dysfunction. Anti-IgE antibodies (including the clinical drug omalizumab) also significantly alleviated angiotensin II-induced cardiac remodeling. Bone marrow transplantation experiments indicated that IgE-induced cardiac remodeling was mediated through non-bone marrow-derived cells. FcεR1 was found to be expressed in both cardiomyocytes and CFs. In cultured rat cardiomyocytes, IgE-induced cardiomyocyte hypertrophy and hypertrophic marker expression were abolished by depleting FcεR1. In cultured rat CFs, IgE-induced CF activation and matrix protein production were also blocked by FcεR1 deficiency. RNA sequencing and signaling pathway analyses revealed that transforming growth factor-β may be a critical mediator, and blocking transforming growth factor-β indeed alleviated IgE-induced cardiomyocyte hypertrophy and cardiac fibroblast activation in vitro.

CONCLUSIONS: Our findings suggest that IgE induction plays a causative role in pathological cardiac remodeling, at least partially via the activation of IgE-FcεR1 signaling in cardiomyocytes and CFs. Therapeutic strategies targeting the IgE-FcεR1 axis may be effective for managing IgE-mediated cardiac remodeling.

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Clinical Perspective

What Is New?

- High serum immunoglobulin E (IgE) levels are correlated with cardiac remodeling and heart failure in humans and mice.
- Blocking IgE-FcεR1 signaling through genetic depletion of FcεR1 or anti-IgE antibodies suppresses angiotensin II- or transverse aortic constriction-induced cardiac remodeling.
- IgE directly stimulates cardiomyocyte hypertrophy and cardiac fibroblast activation depending on FcεR1 in vitro.

What Are the Clinical Implications?

- IgE and its receptor FcεR1 may be novel potential therapeutic targets for treating cardiac remodeling, especially for the patients with heart failure combined with allergy.
- Omalizumab may be an effective drug for patients with cardiac failure, especially for those with a history of allergy.

Hear failure (HF), the end stage of many cardiac diseases, continues to be a life-threatening disorder with high morbidity and mortality. Over the past decades, more patients with HF benefited from the improved clinical treatments; however, the outcome often remains unsatisfactory.¹ Cardiac remodeling is recognized as a determinant of the clinical outcomes of HF,² characterized by cardiomyocyte hypertrophy and cardiomyocyte death, as well as cardiac fibroblast (CF) activation and extracellular matrix deposition.^{3,4} Cardiomyocyte hypertrophy develops when the heart responds to chronic stresses such as pressure overload,⁵ inflammation,⁶ ischemic injury,⁷ and neurohormonal overactivation.⁸ CFs can be activated by various genetic, biomechanical, and humoral factors, as well as undergo phenotype changes from fibroblasts to myofibroblasts capable of proliferating, migrating, and producing extracellular matrix.⁹ Previous studies have shown that transforming growth factor-β (TGF-β),¹⁰ calcium channel regulators,¹¹ matrix metalloproteinases,¹² and contractile proteins¹³ contribute to HF. Several lines of evidence from animal and human studies have identified multiple risk factors of HF, such as hypertension and coronary artery disease. There is great interest in continuing identification of unknown risk factors for HF, which may facilitate the development of novel therapeutic strategies for HF intervention.

Immunoglobulins, as major host proteins in plasma, function as antibacterial and antiviral agents and strengthen cell phagocytosis.^{14,15} There are multiple classes of immunoglobulins, including IgG, IgA,

IgM, IgD, and IgE.¹⁶ IgE belongs to a class of immunoglobulins involved in immune response to specific allergens.¹⁷ Allergies are the most common cause of elevated IgE. Several epidemiological studies have found increased risk of coronary heart disease and HF among patients with allergic diseases such as asthma.^{18–21} We and others have previously found that high IgE levels are associated with several types of vascular disorders, such as atherosclerosis and abdominal aortic aneurysm.^{22,23} The expression of the IgE receptor FcεR1 has been observed in mast cells, macrophages, CD4⁺ T cells, smooth muscle cells, and endothelial cells.^{22,23} IgE promotes inflammation and vascular remodeling, which is FcεR1-dependent.^{22,23} However, it remains unknown whether immunoglobulins are involved in HF, and particularly whether IgE and its receptor FcεR1 are expressed in cardiac cells and contribute to HF.

Recently, we found that serum IgE levels are significantly elevated in patients with HF compared with volunteers without HF. We therefore explored the novel role of IgE in pathological cardiac remodeling as well as the underlying mechanism. We examined the expression and changes of IgE-FcεR1 signaling in human failing hearts and mouse diseased hearts induced by transverse aortic constriction (TAC) and angiotensin II (Ang II) infusion. We investigated the effects of IgE-FcεR1 blockade on cardiac function and cardiac remodeling with genetically engineered mice or IgE antibodies. More important, we showed the direct actions of IgE-FcεR1 on isolated cardiomyocytes and CFs and characterized the potential mechanisms by unbiased RNA sequencing analyses. This work extends our understanding of the role of IgE and FcεR1 in cardiac cells and provides new potential therapeutic targets to prevent pathological cardiac remodeling.

METHODS

Further detailed information about methods is available in the [Methods in the Data Supplement](#). The authors declare that all supporting data are available within the article and in the [Data Supplement](#).

Patient and Population Study

Patients with HF with reduced ejection fraction and the age- and sex-matched patients without HF were enrolled in a single-center clinical cohort from January to June 2019 in Peking Union Medical Hospital. For this study, 60 patients with HF with reduced ejection fraction and 72 subjects without HF were enrolled. The Institutional Ethics Committee of Peking Union Medical College approved the investigation. Data from the NHANES database (National Health and Nutrition Examination Survey) of the civilian, noninstitutionalized population of the United States (2005–2006) were used for this study. A detailed description of the laboratory methods can

be found on the NHANES web page (http://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/AL_IGE_D.htm).

Animals

FcεR1 knockout (FcεR1-KO) male mice (C57BL/6J, N9) were from The Jackson Laboratory, Bar Harbor, ME. C57BL/6J wild-type (WT) mice were purchased from Beijing Vital River Laboratory Animal Technology Co, Ltd. Experimental mice were littermates with a syngeneic C57BL/6J background. For the TAC-induced cardiac remodeling mouse model, 9-week-old WT male mice were subjected to TAC by left ventricular (LV) pressure overload for 8 weeks, as previously described.²⁴ For the cardiac remodeling model induced by Ang II, 9-week-old WT male mice were implanted with osmotic pumps (Alzet MODEL 2002; DURECT, Cupertino, CA) releasing Ang II (1000 ng/kg per min) for 14 days. In the IgE-FcεR1 blockage model, KO mice and the clinical drug omalizumab (100 µg/dose per mouse, every 1 week, total 2 doses, subcutaneous injection) were used for experiments. Furthermore, anti-IgE antibody (2 µg/dose per mouse, every 3 days, total 5 doses, tail vein injection) was used to block IgE-FcεR1 in the Ang II-induced mice. IgE or heat-inactivated IgE (2.5 µg/dose per mouse, once every 3 days for 4 weeks, tail vein injection) was used to explore the effects of IgE in vivo. Inactivated IgE was prepared by boiling for 5 minutes.²² All surgical procedures were performed in accordance with the standards of the National Institutes of Health and approved by the Animal Subjects Committee of Peking Union Medical College.

Bone Marrow–Transplanted Chimeric Mice

Bone marrow (BM) transplantation assays were performed as described previously.²⁵ Chimeric mice were generated using the following procedures: briefly, sex- and age-matched WT mice and FcεR1-KO mice were selected. The BM cells were extracted from donor mice by flushing the femurs and tibias with PBS and were subsequently injected into recipient mice (1×10^7 cells/mouse, tail vein injection) irradiated with 800 cGy x-rays. The mice were then cultured in a clean environment with acidified antibiotic water and sterilized food. Seven days later, the chimeric mice were infused with Ang II (1000 ng/kg/min) for 14 days to induce cardiac remodeling.

Neonatal Rat Cardiac Fibroblasts and Cardiomyocyte Isolation and Culture

As described in previous research,^{26,27} primary neonatal rat CFs and cardiomyocytes (2–3 days old) were prepared. Briefly, neonatal rat hearts were digested in 2 mL enzyme solution containing 100 U/mL collagenase type II (Sigma-Aldrich, Deisenhofen, Germany) and 0.5 mg/mL elastase type I (Sigma-Aldrich, Deisenhofen, Germany) at 37°C until the tissue was completely digested. After filtration through a 70 µm sterile nylon filter, the cells were pooled and centrifuged. Then cells were resuspended in DMEM containing 10% (v/v) fetal bovine serum and plated into 10-cm cell culture dishes. After 1 hour, the nonadhered cells (cardiomyocytes) were plated into 6-well plates and incubated for ≈2 days before adenovirus infection or IgE treatment. The attached cells (CFs) were further

incubated in DMEM containing 10% (v/v) fetal bovine serum at 37°C. CFs at passages 2 to 4 were used for all experiments.

RNA Sequencing and Bioinformatic Analysis

After neonatal rat CFs and cardiomyocytes were treated with 5 µg/mL IgE for 24 hours, total RNA was extracted. An NEBNext Ultra RNA library prep kit (NEB, Beijing, China) was used to prepare the ribosomal RNA (rRNA)-depleted RNA sequencing library, and the library was sequenced by Novogene (Novogene Bioinformatics Technology Co, Ltd, Beijing, China). The raw reads were trimmed of adaptors and low-quality sequences, then mapped to the reference genome using HISAT2 software.^{28,29} The read counts of annotated genes were calculated using the GenomicFeatures and GenomicAlignments packages.³⁰ The DESeq2 package³¹ was used to normalize the read counts among samples and to calculate the fragments per kilobase of exon per million reads mapped of genes. Significance tests for differentially expressed genes (DEGs; P value < 0.05) between samples with and without IgE treatment were also performed with the DESeq2 package. Genes with low expression levels (fragments per kilobase of exon per million reads mapped < 1 in all samples) were eliminated. The differentially expressed genes presented in heatmap figures were drawn using the “pHeatmap” R package (<https://rdrr.io/cran/pheatmap/>). The enrichment analyses of gene ontology and KEGG (Kyoto Encyclopedia of Genes and Genomes) were performed using clusterProfiler package.³² The RNA sequencing data could be accessed through the National Center for Biotechnology Information portal with BioProject ID: PRJNA554512.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 8.0 unless otherwise indicated. Data are presented as mean ± SEM. First, the Shapiro-Wilk test was performed to determine whether data were normally distributed. If data were normally distributed, the Student's t test was used to analyze the difference between 2 groups. If the data were not normally distributed, the Mann-Whitney test was chosen to test the difference between 2 groups. Two-way ANOVA with the Bonferroni post hoc test was performed to compare the differences among the multiple groups, and a P value < 0.05 was considered statistically significant.

Statistical analyses of 4671 people from NHANES were performed using SAS (Version 9.4, SAS Institute Inc, Cary, NC). First, comparisons of age, sex, race, smoking status, and diabetes mellitus between subjects with and without HF were performed separately with χ^2 tests. Comparisons of total serum IgE levels between HF and non-HF were performed by Student's t test. Similarly, a total of 60 patients with HF and 72 subjects without HF from Peking Union Medical College Hospital were also analyzed using SAS. Demographic characteristics (sex, smoking status, diabetes mellitus, hypertension, and hyperlipidemia) of the populations with or without HF were analyzed by χ^2 test. Serum IgE was analyzed by Student's t test. Pearson analysis was used to determine the correlation between the serum total IgE level and NT-proBNP. A P value < 0.05 was considered statistically significant.

RESULTS

Elevated Serum IgE and Cardiac FcεR1 Expression Are Correlated With HF in Humans and Mice

To test whether the elevated serum IgE is a common phenomenon in HF, we analyzed the immunoglobulin profiles in patients with HF (Table I in the Data Supplement). Significant increases in IgE (Figure 1A) and IgA (Figure IA in the Data Supplement) were found in patients with HF compared with the non-HF controls, but not so for IgG and IgM (Figure IB and IC in the Data Supplement). This result suggests that an elevated serum level of IgE is correlated with HF. In addition to IgE, the IgE receptor, FcεR1, was also significantly upregulated in heart tissues of the HF group compared with the non-HF group (Figure 1B). To explore the clinical significance of elevated serum IgE levels, Pearson correlation analysis was conducted between IgE and N-terminal pro-B-type natriuretic peptide (NT-proBNP, a biochemical marker of HF). As shown in Figure 1C, serum IgE levels were significantly correlated with N-terminal pro-B-type natriuretic peptide (NT-proBNP). Furthermore, serum IgE levels were the highest in New York Heart Association grade III HF (Figure ID in the Data Supplement).

We further evaluated the association between IgE levels and human HF using the data from the NHANES collected from 2005 to 2006. Statistical analysis of the data from 4671 participants showed that the mean total serum IgE levels were significantly elevated in patients with HF (225.54 kU/L) compared with the controls without HF (132.00 kU/L; Table II in the Data Supplement). These results imply the association between increased IgE levels and HF.

IgE levels were further assessed in mice subjected to TAC for 8 weeks or Ang II infusion for 2 weeks via osmotic pumps, which stimulated significant pathological cardiac remodeling or dysfunction (Figure II in the Data Supplement). Similar to the human data, serum IgE levels were significantly elevated in both mouse models (Figure 1D and 1G). However, serum IgA did not change in mouse models (Figure IE and IF in the Data Supplement), which is different from human HF (Figure IA in the Data Supplement). Therefore, we specifically focused on IgE in this study because of the similar changes observed in both human and mouse subjects with HF. In addition, FcεR1 mRNA levels and protein levels are significantly upregulated in heart tissues from TAC- (Figure 1E and 1F) and Ang II-treated mice (Figure 1H and 1I) compared with those from control mice. These data together suggest that elevated serum IgE may be correlated with pathological cardiac remodeling.

FcεR1 Deficiency Ameliorates TAC- and Ang II-Induced Pathological Cardiac Remodeling In Vivo

Next, we attempted to determine the role of IgE-FcεR1 in pathological cardiac remodeling and HF in vivo through global KO of FcεR1 in mice. We first tested the TAC model, a well-established mouse HF model. There were no blood pressure differences observed between WT control mice (FcεR1-WT) and FcεR1 KO mice (FcεR1-KO; data not shown). In FcεR1-WT mice, TAC for 8 weeks significantly increased heart size (Figure IIIB in the Data Supplement) and the ratio of heart weight to body weight (Figure 2A) compared with sham FcεR1-WT controls. These changes by TAC were significantly attenuated in FcεR1-KO mice (Figure IIIB in the Data Supplement and Figure 2A). Echocardiography was performed to monitor the progression of cardiac structure and functional changes (Table III in the Data Supplement). After TAC, FcεR1-WT mice exhibited a reduction of ejection fraction and fractional shortening, which was significantly improved in FcεR1-KO mice (Figure 2B, Figure IIC and Table III in the Data Supplement). In addition, LV internal diameter, LV anterior wall and LV posterior wall thickness were significantly reduced in FcεR1-KO mice (Table III in the Data Supplement).

We performed histological and biochemical analyses to further evaluate myocardial hypertrophy and fibrosis in heart tissues. TAC induced a significant increase in cross-sectional area (CSA) assessed by wheat germ agglutinin staining in FcεR1-WT hearts, which was significantly reduced in FcεR1-KO hearts (Figure 2C and 2D). TAC-induced upregulation of prohypertrophic markers atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) were also significantly decreased in FcεR1-KO hearts (Figure IIID in the Data Supplement). Sirius Red staining revealed that fibrosis was significantly reduced in FcεR1-KO hearts compared with FcεR1-WT hearts (Figure 2E and 2F). Consistently, the protein expression of collagen 1 (a fibrotic protein) was significantly lower in TAC-treated FcεR1-KO mice (Figure 2G). Periostin³³ and α-smooth muscle actin (α-SMA),³⁴ the markers of fibroblast activation and transdifferentiation to myofibroblast phenotype, were also significantly decreased in FcεR1-KO mice compared with FcεR1-WT, as assessed by quantitative polymerase chain reaction (qPCR; Figure IIIE in the Data Supplement) and immunostaining (Figure IIIF–IIII in the Data Supplement).

We further confirmed the role of FcεR1 in the cardiac remodeling model induced by Ang II infusion. We obtained similar results, showing that Ang II-induced pathological cardiac remodeling is significantly attenuated in FcεR1-KO mice compared with FcεR1-WT mice, including the decrease of the ratio of

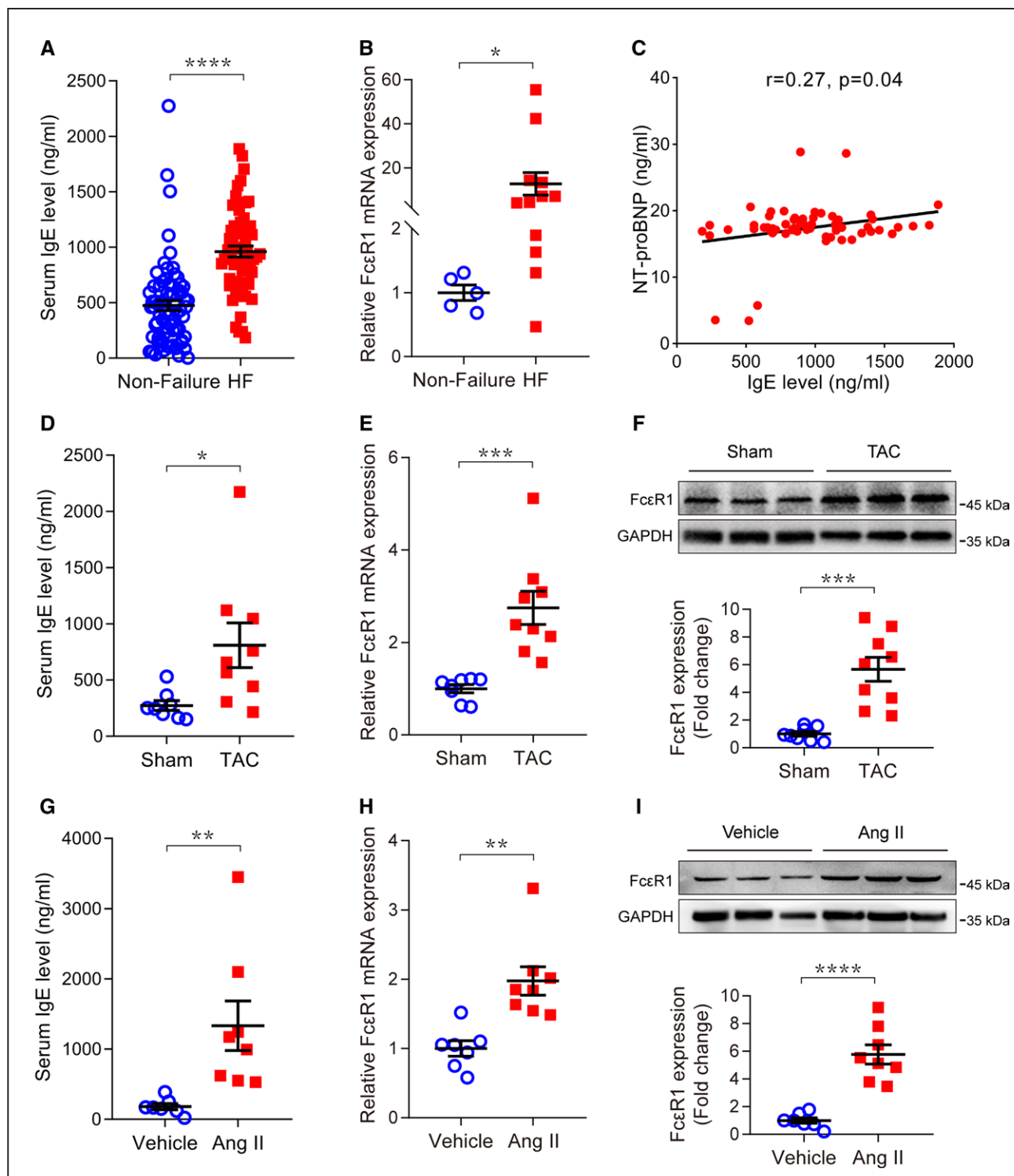


Figure 1. The levels of immunoglobulin E (IgE) and its receptor FcεR1 in patients with heart failure (HF) and stress-induced cardiac remodeling mice. **A**, ELISA analysis was used in the detection of serum IgE in patients with HF (n=60) and age/sex-matched nonfailure subjects (n=72). **B**, Quantification of quantitative polymerase chain reaction (qPCR) analysis of FcεR1 mRNA in the heart tissue of human patients with HF (n=12) and nonfailure subjects (n=5; fold change vs nonfailure subjects). **C**, Pearson correlation analysis of the relationship between serum IgE and serum NT-proBNP in patients with HF (n=60). **D** through **F**, Serum IgE levels (**D**), qPCR quantification of FcεR1 mRNA (fold change vs sham; **E**), immunoblot analysis of FcεR1 protein expression (**upper**) and quantification of the FcεR1 protein level normalized to GAPDH protein (fold change vs sham; **lower**; **F**), in C57BL/6J mice for 8 weeks after transversus aortic constriction (TAC, n=9) or sham (n=8) operation. **G** through **I**, Serum IgE levels (**G**), qPCR quantification of FcεR1 mRNA (fold change vs vehicle; **H**), immunoblot analysis of FcεR1 protein expression (**upper**) and quantification of the FcεR1 protein level normalized to GAPDH protein (fold change vs vehicle; **lower**; **I**), in C57BL/6J mice treated with vehicle (n=7) or 1000 ng/kg per min angiotensin II (Ang II; n=8) for 2 weeks. Error bars represent SEM. The results are shown as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, using Student's *t* test (**A**, **D**–**I**) or Mann-Whitney test (**B**). NT-proBNP indicates N-terminal pro-B-type natriuretic peptide.

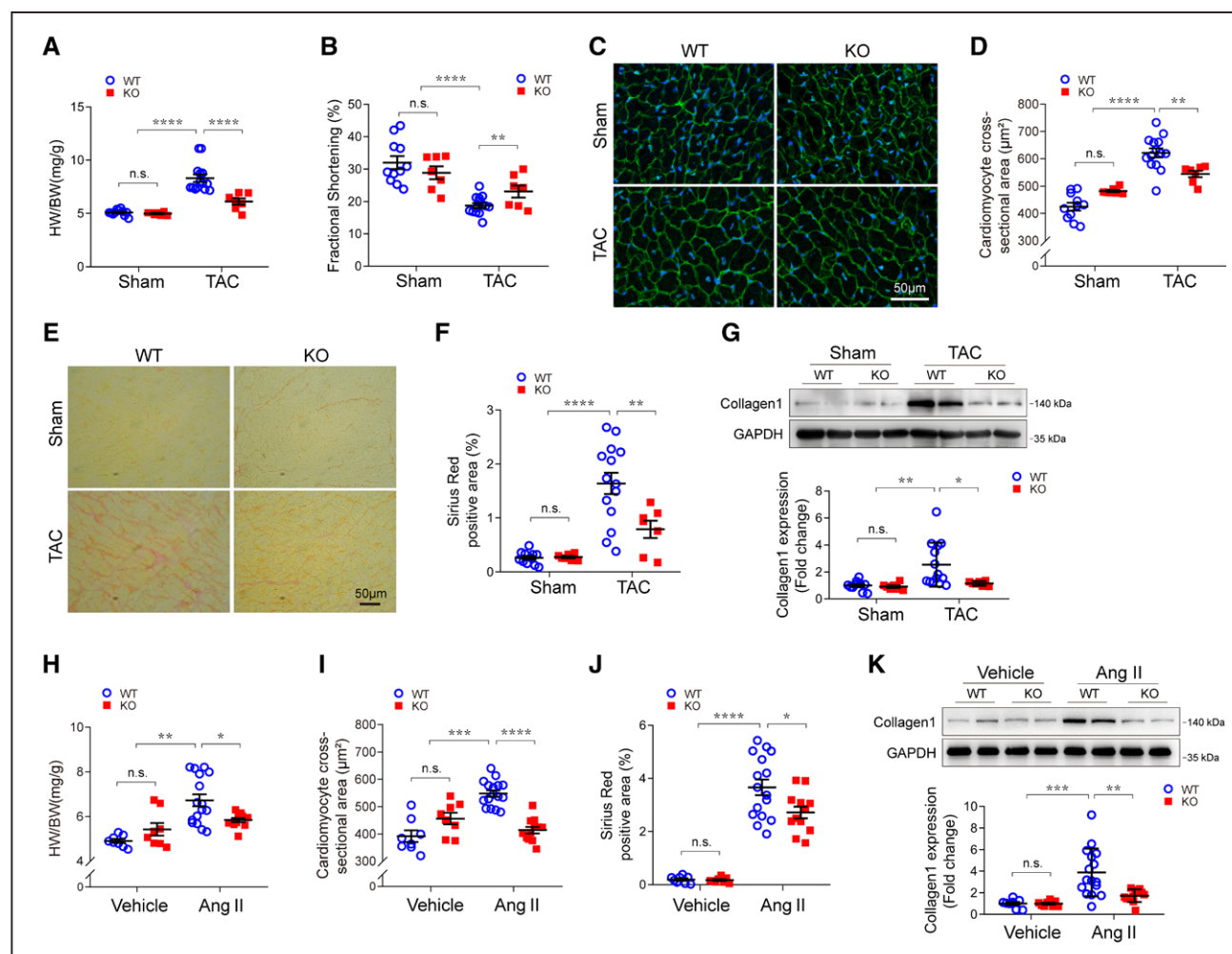


Figure 2. Effects of FcεR1 deficiency on transverse aortic constriction (TAC)-induced and angiotensin II (Ang II)-induced cardiac remodeling mice. **A**, Statistical analysis of heart to body weight ratio after 8-week treatment with sham or TAC in FcεR1-wild-type (WT) and FcεR1 knockout (FcεR1-KO) mice. **B**, Left ventricular fractional shortening assessed by echocardiography. **C**, Immunohistochemistry staining of cardiomyocytes in the left ventricular sections (6 μm) from the 4 indicated treatment groups. Cardiomyocytes were stained with WGA (green), and nuclei were stained with DAPI (blue). Scale bars, 50 μm. **D**, Quantification of the data from the WGA staining. A total of 450 cells per mouse randomly selected from 9 fields in 3 sections (3 random fields from each section) were measured. **E**, Representative images of Sirius Red staining. Scale bars, 50 μm. **F**, Quantification of the above Sirius Red staining. A total of 9 randomly selected images from 3 sections per mouse (3 images from each section) in the indicated 4 groups were analyzed. **G**, **Top**, Representative Western blot analysis of collagen 1 and GAPDH in whole cardiac tissue from the FcεR1-WT and FcεR1-KO mice treated with sham or TAC. **Bottom**, Statistical analysis of collagen 1 expression normalized to GAPDH in 4 indicated groups (fold change vs sham-WT group). **H**, Statistical analysis of heart to body weight ratio after 2-week treatment of vehicle or Ang II in FcεR1-WT and FcεR1-KO mice. **I**, Quantification analysis of the data from the WGA staining. **J**, Quantification analysis of the Sirius Red staining. **K**, **Top**, Representative Western blot analysis of collagen 1 and GAPDH in whole cardiac tissue from FcεR1-WT and FcεR1-KO mice treated with vehicle or Ang II. **Bottom**, Statistical analysis of collagen 1 expression normalized to GAPDH (fold change vs vehicle-WT group). Total n=11 (sham-WT), n=7 (sham-KO), n=14 (TAC-WT), or n=7 (TAC-KO) per group; n=8 (vehicle-WT), n=8 (vehicle-KO), n=16 (Ang II-WT) or n=12 (Ang II-KO) per group. The results are shown as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, n.s. indicates no significance in 2-way ANOVA with Bonferroni post hoc test. BW indicates body weight; DAPI, 4',6'-diamidino-2-phenylindole; HW, heart weight; and WGA, wheat germ agglutinin.

heart weight to body weight (Figure 2H), myocardial CSA (Figure IIIJ in the Data Supplement, Figure 2I), and the expression of prohypertrophic markers ANP and BNP (Figure IIJK in the Data Supplement). In addition, cardiac interstitial fibrosis (Figure IIIL in the Data Supplement, Figure 2J), collagen 1 expression (Figure 2K), and expression of the fibrotic markers periostin and α-SMA (Figure IIIM–IIIQ in the Data Supplement) were also significantly lower in FcεR1-KO than FcεR1-WT mice. These findings together suggest that IgE-FcεR1 plays a critical role in pathological cardiac remodeling or dysfunction.

Anti-IgE Antibodies Prevent and IgE Administration Promotes Pathological Cardiac Remodeling In Vivo

To investigate the potential therapeutic possibility of IgE blockage in cardiac remodeling, we evaluated the effects of the clinical IgE antagonist omalizumab, an anti-IgE monoclonal antibody, on Ang II-induced cardiac remodeling (Figure 3A). We observed a significant decrease in serum IgE in Ang II-infused mice after omalizumab treatment (Figure IV in the Data Supplement). Compared with the saline group, Ang

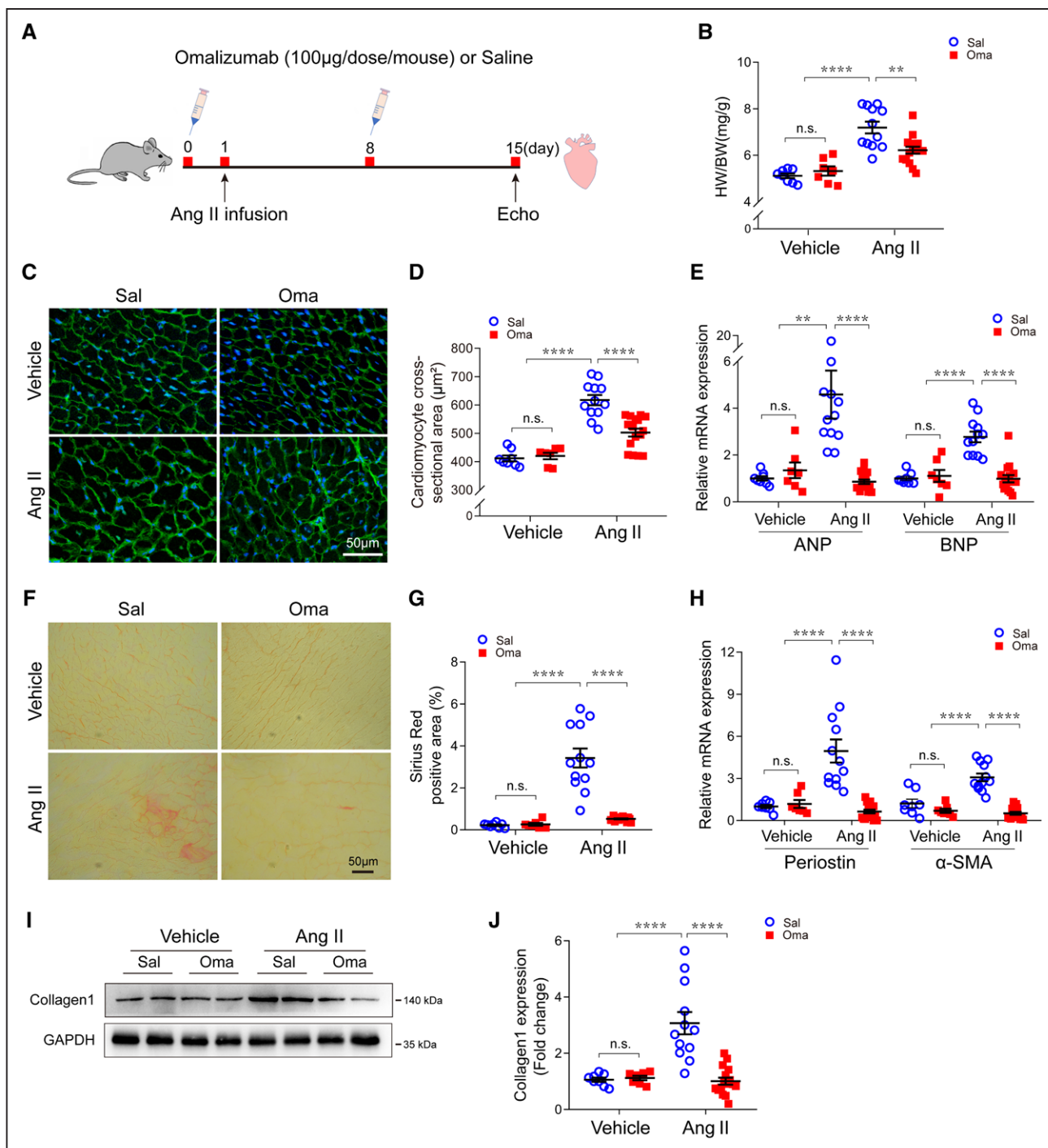


Figure 3. Effects of omalizumab on Ang II-induced cardiac remodeling.

A, Experimental design for assessing the effect of omalizumab on Ang II-induced cardiac remodeling. A total of 100 µg omalizumab was injected intraperitoneally 1 day before Ang II infusion. The second injection of omalizumab (100 µg) or saline (100 µL) was applied 7 days after Ang II treatment. **B**, Statistical analysis of heart to body weight ratio. **C**, Wheat germ agglutinin (green) and 4'-diamidino-2-phenylindole (blue) staining of cardiac sections (6 µm) from the indicated mice. Scale bars, 50 µm. **D**, Comparison of cardiomyocyte area among the 4 indicated groups. A total of 450 cells per mouse randomly selected from 9 fields in 3 sections (3 random fields from each section) were measured. **E**, Analysis of ANP and BNP mRNA relative expression (fold change vs vehicle-saline group) measured by quantitative polymerase chain reaction (qPCR). **F**, Sirius Red staining of interstitial collagen deposition in hearts from mice treated with omalizumab or saline. Scale bars, 50 µm. **G**, Quantification of fibrotic area shown as a percentage of total area. A total of 9 fields from 3 sections (3 fields from each section) per mouse were randomly selected for analysis. **H**, qPCR analysis of periostin and α -smooth muscle actin (α -SMA) mRNA expression (fold change vs vehicle-saline group). **I**, Western blot analysis of collagen 1 and GAPDH expression in the cardiac tissue from mice under the indicated treatments. **J**, Statistical analysis of the collagen 1 relative expression normalized to GAPDH (fold change vs vehicle-saline group). Total n=8 (vehicle-saline), n=7 (vehicle-Oma), n=12 (Ang II-saline), or n=16 (Ang II-Oma) per group. The results are shown as mean $\pm$ SEM. ** $P < 0.01$, **** $P < 0.0001$, n.s. indicates no significance in 2-way ANOVA with Bonferroni post hoc test. Ang II indicates angiotensin II; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; BW, body weight; DAPI, 4'-diamidino-2-phenylindole; Echo, echocardiography; HW, heart weight; Oma, omalizumab; Sal, saline; and WGA, wheat germ agglutinin.

II-induced cardiac remodeling was markedly inhibited by omalizumab (100 µg omalizumab once/week), reflected by the decreased ratio of heart weight to body weight (Figure 3B), myocardial CSA (Figure 3C and 3D), and the expression of prohypertrophic markers ANP and BNP (Figure 3E). Furthermore, omalizumab treatment significantly inhibited the cardiac interstitial fibrosis (Figure 3F and 3G), concomitantly with a decrease in periostin and α -SMA expression assessed by qPCR (Figure 3H) and immunostaining (Figure VA–VD in the Data Supplement), as well as collagen 1 expression (Figure 3I and 3J).

We also tested a different mouse anti-IgE antibody (Figure VIA in the Data Supplement). Similar to the omalizumab, the mouse anti-IgE antibody significantly reduced serum IgE (Figure VIB in the Data Supplement) and alleviated the Ang II-induced cardiac remodeling, reflected by the decreased ratio of heart weight to body weight (Figure VIC in the Data Supplement), myocardial CSA (Figure VID and VIE in the Data Supplement), and prohypertrophic markers ANP and BNP (Figure VIF in the Data Supplement), cardiac interstitial fibrosis (Figure VIG and VIH in the Data Supplement), the expression of cardiac fibroblast activation markers periostin and α -SMA (Figure VIIM in the Data Supplement), as well as collagen 1 expression (Figure VIN in the Data Supplement).

To further detect whether IgE administration alone can promote pathological cardiac remodeling and dysfunction, we administered IgE or heat-inactivated IgE (2.5 µg/dose per mouse) to Fc ϵ R1-WT and Fc ϵ R1-KO mice via tail-vein injection, once every 3 days for 4 weeks (Figure VIIA in the Data Supplement). As anticipated, the serum IgE levels were significantly elevated in IgE-treated WT mice (853.13±81.90 ng/mL) and KO mice (829.74±114.82 ng/mL). We found that IgE administration significantly increased the ratio of heart weight to body weight compared with inactivated IgE in WT animals, and this finding was significantly blunted in Fc ϵ R1-KO mice (Figure VIIB in the Data Supplement). Echocardiographic measurements showed IgE apparently reduced ejection fraction and fractional shortening in Fc ϵ R1-WT mice (Table IV in the Data Supplement). In addition, LV anterior wall and LV posterior wall thickness as well as LV mass were also significantly increased in IgE-treated WT mice, whereas these effects were reversed in Fc ϵ R1-KO mice (Table IV in the Data Supplement). Histological and biochemical analyses showed IgE-treated WT mice developed cardiac hypertrophy and fibrosis as indicated by an increase in the CSA of myocytes (Figure VIIC and VIID in the Data Supplement), Sirius Red–positive area (Figure VIIIE and VIIF in the Data Supplement), and collagen 1 expression (Figure VIIG and VIIH in the Data Supplement), whereas these changes were abrogated in IgE-administrated Fc ϵ R1-KO mice. There

was no significant adverse effect on liver and kidney function in mice after IgE administration (Table V in the Data Supplement). These results together indicate a predominant role of IgE in cardiac remodeling and suggest a potential novel application of clinical drugs targeting IgE in treating cardiac diseases associated with pathological cardiac remodeling.

The Effect of IgE on Ang II–Induced Cardiac Remodeling Is Not Mediated by BM-Derived Cells

To determine the cell types involved in IgE-Fc ϵ R1–mediated cardiac remodeling, chimeric mice were generated with BM transplantation as previously described.¹⁹ Both Fc ϵ R1^{+/+} (WT) and Fc ϵ R1^{-/-} (KO) recipient mice were transplanted with the BM from either Fc ϵ R1^{+/+} (wt) or Fc ϵ R1^{-/-} (ko) donor mice. One week after BM transplantation, the chimeric mice were infused with Ang II for 2 weeks (Figure 4A). Various parameters of cardiac remodeling were analyzed, including the ratio of heart weight to body weight (Figure 4B), myocardial CSA (Figure 4C and 4D), prohypertrophic markers ANP and BNP (Figure 4E), as well as cardiac interstitial fibrosis (Figure 4F and 4G), fibrotic markers periostin and α -SMA (Figure 4H, Figure VIIIA–VIIID in the Data Supplement) and collagen 1 (Figure 4I and 4J). All these parameters showed no significant differences in comparisons between the WT/wt versus WT/ko or KO/wt versus KO/ko treatment groups. However, these markers were significantly reduced in KO/wt and KO/ko compared with their levels in WT/wt and WT/ko mice. Taken together, these results suggest that the genotype of BM-derived cells does not have a significant overall effect on cardiac remodeling.

IgE Directly Promotes Cardiomyocyte Hypertrophy, CF Activation, and Matrix Protein Production

To further examine whether IgE can directly function in cardiac cells, we first examined the expression of Fc ϵ R1 in cardiomyocytes. As shown in Figure 5A, Fc ϵ R1 protein expressed in rat neonatal cardiomyocytes and mast cells that were previously shown to express Fc ϵ R1 were used as positive controls.^{35,36} We subsequently examined whether IgE-Fc ϵ R1 induces cardiomyocyte hypertrophy using adenovirus expressing short hairpin RNA (shRNA) specifically targeting Fc ϵ R1 (sh-Fc ϵ R1) or scrambled shRNA in neonatal rat cardiomyocytes (Figure IXA and IXB in the Data Supplement). As anticipated, IgE strongly induced hypertrophy in control cardiomyocytes, reflected by the increased cardiomyocyte area assessed by F-actin immunofluorescence staining with ActinRed 555 reagent (Figure 5B and 5C),

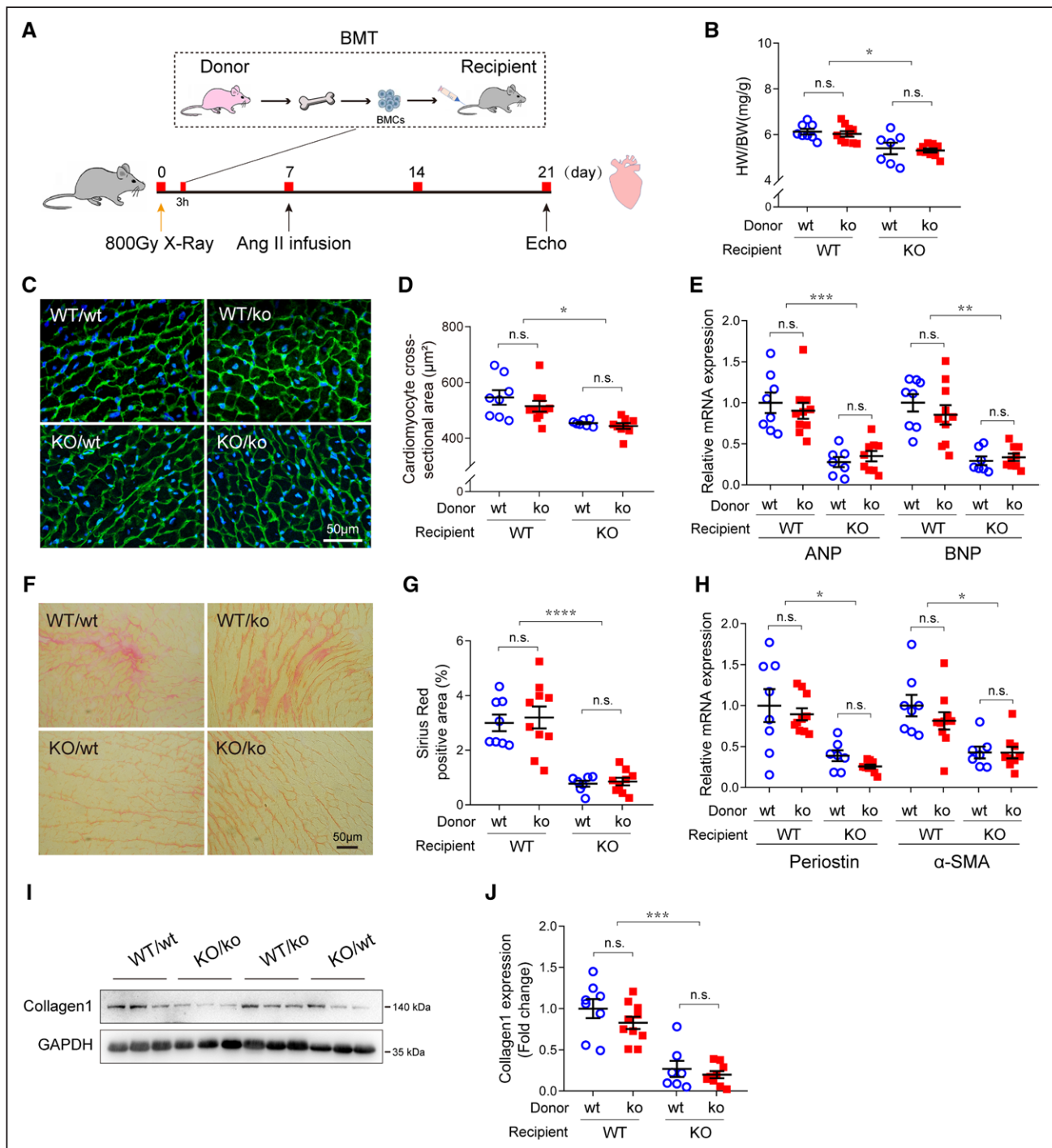


Figure 4. Effects of bone marrow–derived cells expressed FcεR1 on Ang II–induced cardiac remodeling.

A, Pipeline for the bone marrow transplantation experiment to assess the effect of bone marrow–derived FcεR1–positive cells on cardiac remodeling. **B**, Quantification of heart to body weight ratio in the indicated groups of mice. **C**, WGA (green) and DAPI (blue) staining of mouse cardiac sections (6 μm) from the mice in the indicated groups. Scale bars, 50 μm. **D**, Quantification of the cardiomyocyte area shown in the above WGA and DAPI staining. A total of 450 cells per mouse randomly selected from 9 fields in 3 sections (3 random fields from each section) were measured. **E**, qPCR quantification of atrial natriuretic peptide and B-type natriuretic peptide mRNA relative expression (fold change vs WT/wt group). **F**, Representative images of Sirius Red staining of left ventricular sections from the indicated groups. Scale bars, 50 μm. **G**, Quantification of interstitial fibrosis in mice from WT or KO bone marrow chimeras. A total of 9 fields from 3 sections (3 fields from each section) per mouse were randomly selected for analysis. **H**, qPCR analysis of periostin and α-SMA mRNA expressed in left ventricular sections from the indicated groups (fold change vs WT/wt group). **I**, Western blot analysis of collagen 1 and GAPDH expression in the cardiac tissue from mice under the indicated treatments. **J**, Statistical analysis of the collagen 1 relative expression normalized to GAPDH (fold change vs WT/wt group). Total n=8 (WT/wt), n=10 (WT/ko), n=7 (KO/wt), or n=9 (KO/ko) per group. The results are shown as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, n.s. indicates no significance in 2-way ANOVA with Bonferroni post hoc test. α-SMA indicates α-smooth muscle actin; Ang II, angiotensin II; ANP, atrial natriuretic peptide; BMT, bone marrow transplantation; BNP, B-type natriuretic peptide; BW, body weight; DAPI, 4′,6-diamidino-2-phenylindole; Echo, echocardiography; HW, heart weight; KO, knockout; qPCR, quantitative PCR; WGA, wheat germ agglutinin; and WT, wild-type.

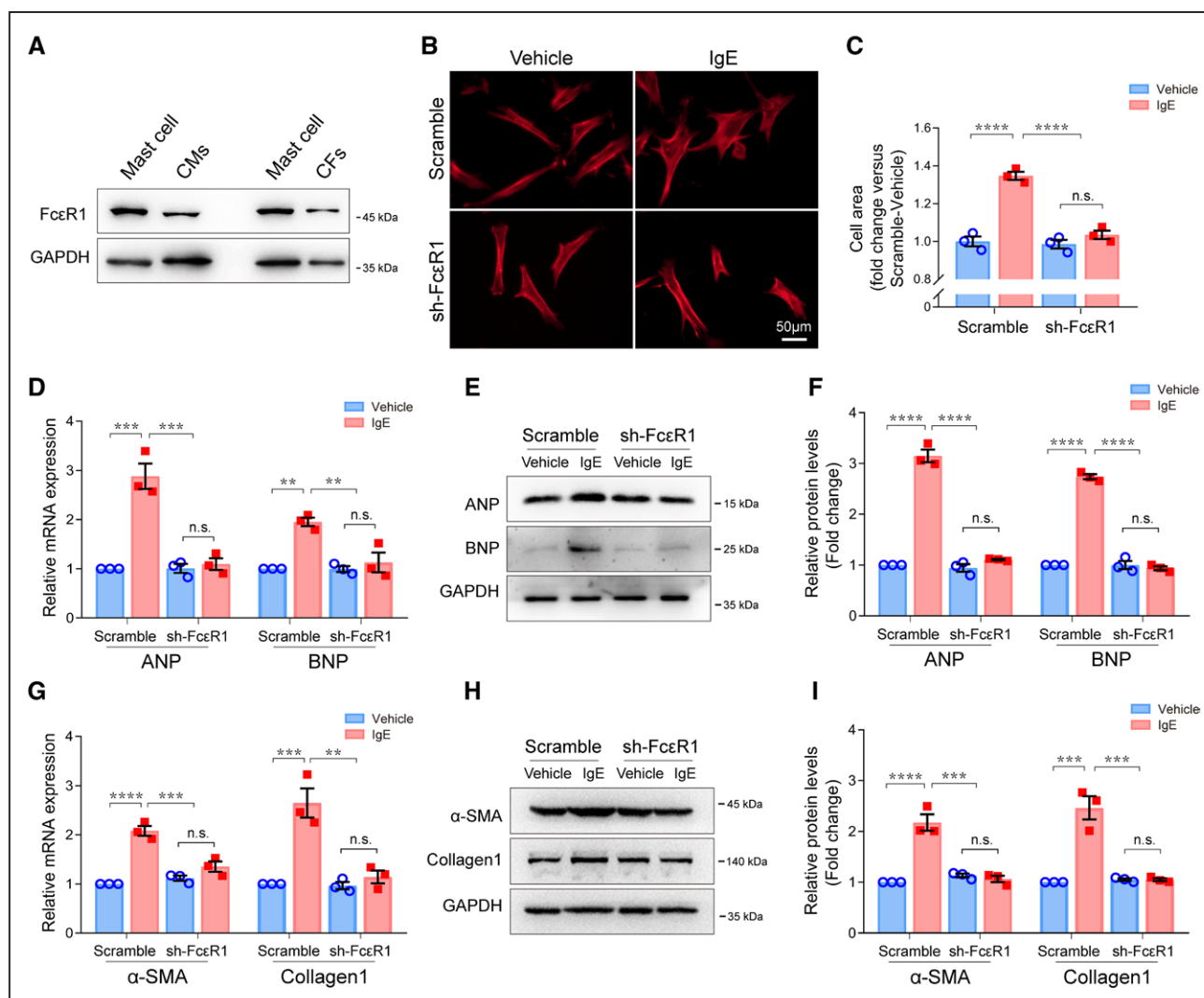


Figure 5. Effects of IgE treatment on rat cardiomyocytes and cardiac fibroblasts in vitro.

A, Representative Western blot of FcεR1 expression in neonatal rat cardiomyocytes and cardiac fibroblasts. Mast cells were used as the positive control. **B**, Representative images of F-actin staining of cardiomyocytes with ActinRed 555 after treatment with 5 μg/mL IgE for 24 hours. Scale bars, 50 μm. **C**, Quantification of the cardiomyocyte area shown in the above F-actin staining. Randomly selected 180 cardiomyocytes from 3 independent experiments were measured after IgE treatment in cardiomyocytes infected with scramble adenovirus or sh-FcεR1 adenovirus. **D**, qPCR relative expression analysis (fold changes vs vehicle-scramble group) of ANP and BNP mRNA expression in cardiomyocytes treated with the indicated reagents. **E**, Representative Western blot of ANP and BNP expression in cardiomyocytes infected with scramble adenovirus or sh-FcεR1 adenovirus. **F**, Statistical analysis of the ANP and BNP relative expression normalized to GAPDH from 3 independent experiments (fold changes vs vehicle-scramble group). **G**, qPCR analysis of α-SMA and collagen 1 expressed in rat primary fibroblast cells as the indicated treatments (fold changes vs vehicle-scramble group). **H**, Representative Western blot of α-SMA and collagen 1 protein expression in primary rat cardiac fibroblasts after the indicated treatments. GAPDH was used as the reference control. **I**, Statistical analysis of α-SMA and collagen 1 protein relative expression (fold changes vs vehicle-scramble group) from 3 independent experiments. The results are shown as mean±SEM of relative fold changes. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, n.s. indicates no significance in 2-way ANOVA with Bonferroni post hoc test. α-SMA indicates α-smooth muscle actin; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CF, cardiac fibroblast; CM, cardiomyocyte; IgE, immunoglobulin E; and qPCR, quantitative PCR.

and prohypertrophy markers ANP and BNP expression assessed by qPCR (Figure 5D) and Western blots (Figure 5E and 5F). These changes by IgE were significantly attenuated in expressing sh-FcεR1 cardiomyocytes (Figure 5D–5F). These results strongly suggest that IgE directly promotes hypertrophy of cardiomyocytes in an FcεR1-dependent manner.

We also examined the expression of FcεR1 in rat CFs. Similar to cardiomyocytes, FcεR1 was also expressed in rat CFs (Figure 5A). Adenovirus expressing sh-FcεR1 or scrambled shRNA in neonatal rat CFs was used to

explore the role of IgE-FcεR1 in CF activation (Figure IXC and IXD in the Data Supplement). We found that IgE increased the expression of α-SMA (a marker of CF activation) and collagen 1 in CFs (Figure XA–XC in the Data Supplement) in a time-dependent manner. More importantly, IgE-induced up-regulation of α-SMA, and collagen 1 mRNA (Figure 5G) and protein (Figure 5H and 5I) were abolished in CFs with FcεR1 knockdown. These data suggest that IgE directly promotes CF activation and matrix protein production in an FcεR1-dependent manner.

IgE-Regulated Transcriptome Profile and Cardiac Hypertrophy Pathways in Cardiomyocytes

To explore the mechanism by which IgE-FcεR1 regulates cardiomyocyte hypertrophy, the transcriptomes of cardiomyocytes treated with or without IgE were examined using RNA sequencing (Figure 6A). Bioinformatic analysis showed that IgE significantly altered the expression of 1584 genes in cardiomyocytes (Figure 6B). Further gene ontology analysis showed that the IgE-regulated genes are mainly involved in cardiac muscle contraction, muscle tissue development, circulatory system processes, and so forth (Figure 6C, [Table VI in the Data Supplement](#)). KEGG analysis revealed the top 20 KEGG-annotated pathways enriched for the differentially expressed genes stimulated by IgE (Figure 6D, [Table VII in the Data Supplement](#)). Among them, phosphatidylinositol 3 kinase (PI3K)-protein kinase B (AKT) signaling, cGMP-protein kinase G (PKG) signaling, adrenergic signaling, dilated cardiomyopathy, hypertrophic cardiomyopathy, and extracellular matrix receptor interaction pathways have been reported to be involved in cardiac hypertrophy (Figure XI in the [Data Supplement](#)). By overlapping the genes in the above 6 major KEGG pathways with previously reported cardiac hypertrophy-related genes (<https://pathcards.genecards.org/Pathway/2262>), 36 genes were revealed (Figure 6E). We then confirmed the changes of these key genes in IgE-treated cardiomyocytes with or without FcεR1 knockdown by qPCR (Figure 6F). Our results suggest that the adrenergic signaling, TGF-β pathway, PI3K-AKT signaling pathway, and insulin pathway are likely to participate in IgE-induced cardiomyocyte hypertrophy (Figure 6G).

IgE-Regulated Transcriptome Profile and Cardiac Fibrotic Pathways in CFs

Similarly, we performed a whole-transcriptome bioinformatic analysis on IgE-treated CFs (Figure 7A and 7B). Gene ontology analysis indicated that IgE altered the expression of genes responsible for angiogenesis, extracellular matrix organization, steroid metabolic processes, extracellular structure organization, and so forth (Figure 7C, [Table VIII in the Data Supplement](#)). KEGG enrichment analysis revealed that IgE might be involved in TGF-β signal transduction, PI3K-AKT signals, cytokine-cytokine receptor interaction, tight junctions, Hippo signaling pathway, extracellular matrix-receptor interactions, and other related pathways (Figure 7D, [Figure XII in the Data Supplement](#)). The overlapping genes enriched in the major KEGG pathways ([Table IX in the Data Supplement](#)) and critical for cardiac function are shown in Figure 7E. qPCR further confirmed changes of these genes by IgE-FcεR1 in CFs (Figure 7F). Specifically, some well-known fibrotic genes (*Col1a1*,

Col1a2, *Col3a1*, *Col6a1*, and *Col6a2*) were observed to be upregulated by IgE depending on FcεR1 (Figure 7F). In summary, these results suggest that IgE contributes to cardiac fibrosis in CF through regulating genes involved in several fibrotic pathways, such as TGF-β, PI3K-AKT, cytokine-related pathway, and the Hippo signaling pathway (Figure 7G).

IgE-FcεR1 Stimulates TGF-β–Mediated Signaling In Vitro and In Vivo

As TGF-β was found to be upregulated in both cardiomyocytes and CFs by IgE-FcεR1, we further examined the changes in TGF-β mRNA and protein expression in heart tissues from FcεR1-WT or FcεR1-KO mice. We found that both TGF-β mRNA ([Figure XIII A in the Data Supplement](#)) and protein levels (Figure 8A and 8B) were significantly reduced in the hearts of FcεR1-KO mice compared with FcεR1-WT mice after Ang II infusion. Similar results were obtained from the mouse hearts treated with omalizumab ([Figure XIII B in the Data Supplement](#), Figure 8E and 8F) or the mouse anti-IgE antibody ([Figure XIII C–XIII E in the Data Supplement](#)). In addition, we analyzed the expression of TGF-β downstream signaling molecules including PI3K,³⁷ AKT,³⁷ pSmad1/5/9, and pSmad2/3 in vivo. In consistent with change in TGF-β expression, the expression of these core molecules were also significantly reduced in FcεR1-KO mice (Figure 8A–8D), omalizumab-treated mice (Figure 8E–8H), and anti-IgE antibody-treated mice ([Figure XIII D–XIII G in the Data Supplement](#)) compared with control. In contrast, IgE administration increased TGF-β expression ([Figure XIV A and XIV B in the Data Supplement](#)) and activated TGF-β signaling in FcεR1-WT mice, whereas these effects were markedly attenuated in FcεR1-KO mice ([Figure XIV A–XIV D in the Data Supplement](#)).

Next, we sought to confirm the role of TGF-β in IgE-induced cardiomyocyte hypertrophy and CF activation in vitro using TGF-β inhibitor SB431542. The results showed that SB431542 attenuated IgE-induced expression of the prohypertrophic genes ANP and BNP in cardiomyocytes (Figure 8I), and fibrotic genes α-SMA and collagen 1 in CFs (Figure 8J). Furthermore, SB431542 also significantly reduced the expression of TGF-β downstream molecules including PI3K, AKT, pSmad1/5/9, and pSmad2/3 in both cardiomyocytes ([Figure XV A in the Data Supplement](#)) and CFs ([Figure XV B in the Data Supplement](#)). On the basis of these findings, we proposed that IgE promoted cardiac remodeling via directly stimulating cardiac hypertrophy in cardiomyocytes and fibrosis in CFs via TGF-β-PI3K-AKT or TGF-β/Smads signaling pathways. TGF-β is likely to be the most crucial mediator in IgE-FcεR1-induced cardiac remodeling or dysfunction ([Figure XVI in the Data Supplement](#)).

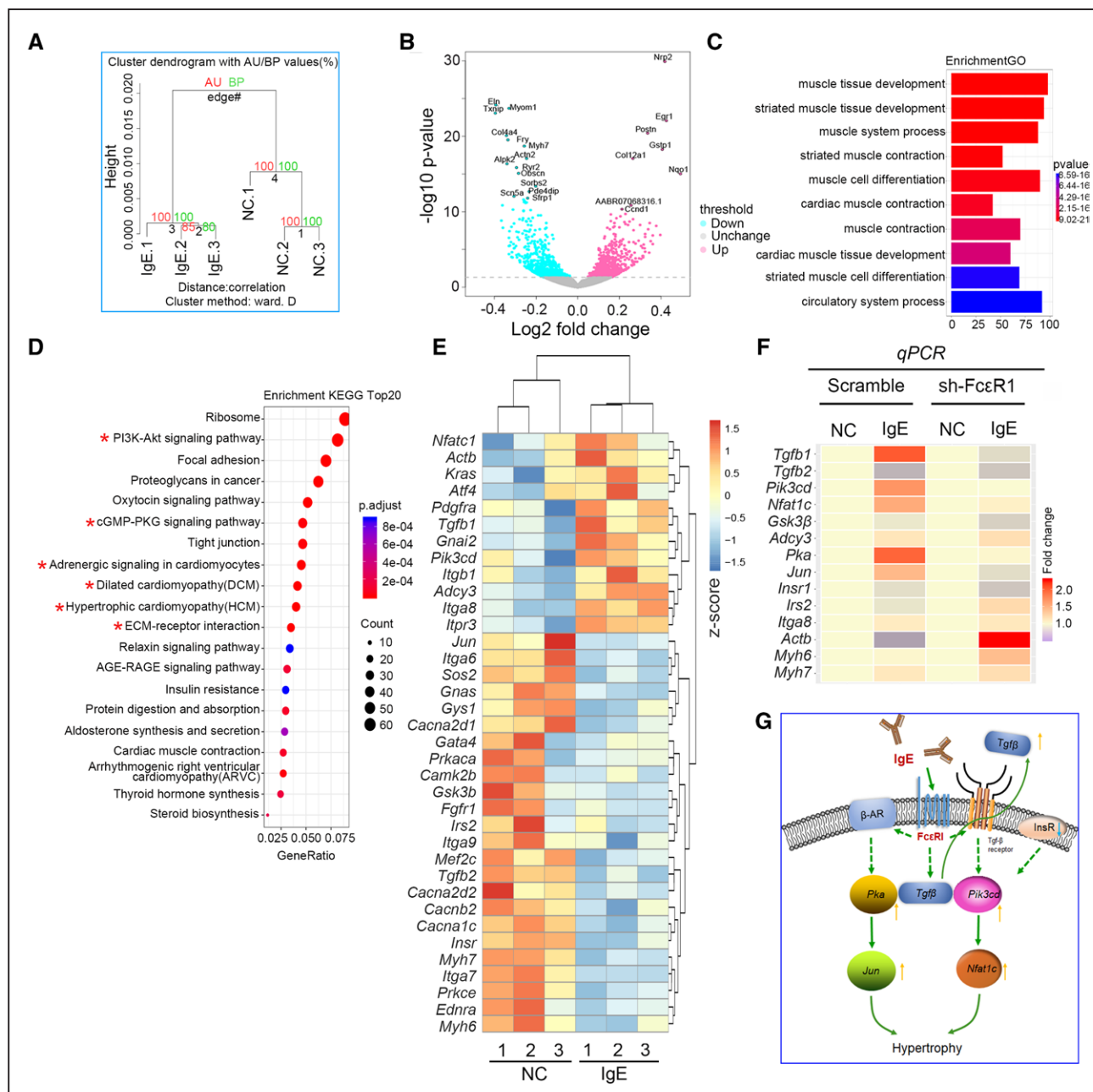


Figure 6. Effects of IgE on transcriptome profile of neonatal rat cardiomyocytes.

A and B. Hierarchical clustering of the RNA sequencing samples on the basis of the gene expression levels (**A**), and the distribution of differentially expressed genes by P value (**B**). The pink dots represent genes with increased expression after IgE treatment, whereas the blue dots represent genes with decreased expression. **C.** GO enrichment categories containing differentially expressed genes in response to IgE. **D.** Top 20 potential pathways in IgE-stimulated cardiomyocytes, annotated by KEGG analysis. **E.** Heatmap showed the key differential hypertrophic related genes which enriched in the 6 pivotal pathways (marked with the red asterisk) from **D**. **F.** qPCR analysis of the core factors in KEGG hypertrophy-related pathways in cardiomyocytes infected with scramble adenovirus (scramble) or sh-FcεR1 adenovirus (sh-FcεR1) in response to IgE. **G.** Proposed model for the effects of IgE on cardiac hypertrophy. AGE-RAGE, advanced glycation end products-receptor for AGE; AU, approximately unbiased; β-AR, β-adrenergic receptor; BP, bootstrap probability; ECM, extracellular matrix; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, gene ontology; IgE, immunoglobulin E; InsR, insulin receptor; NC, negative control; PKG, protein kinase G; qPCR, quantitative PCR; Tgf-β, transforming growth factor-β; and ward.D, Ward's minimum variance method.

DISCUSSION

In the current study, we investigated the potential relationship of IgE and its receptor FcεR1 in cardiac remodeling in both humans and mice. We found the up-regulation of serum IgE and cardiac FcεR1 expression in human HF patients and in mice with cardiac diseases induced by neurohormonal overactivation (such as Ang II)

or chronic pressure overload by TAC. Importantly, using in vitro and in vivo approaches, we demonstrated that IgE promoted cardiac remodeling primarily through effects on cardiomyocytes and CFs through FcεR1. TGF-β appears to be a critical mediator for IgE-FcεR1 in promoting cardiac hypertrophy and fibrosis. In addition, administration of IgE directly activated TGF-β signaling, and promoted pathological cardiac remodeling (Figures

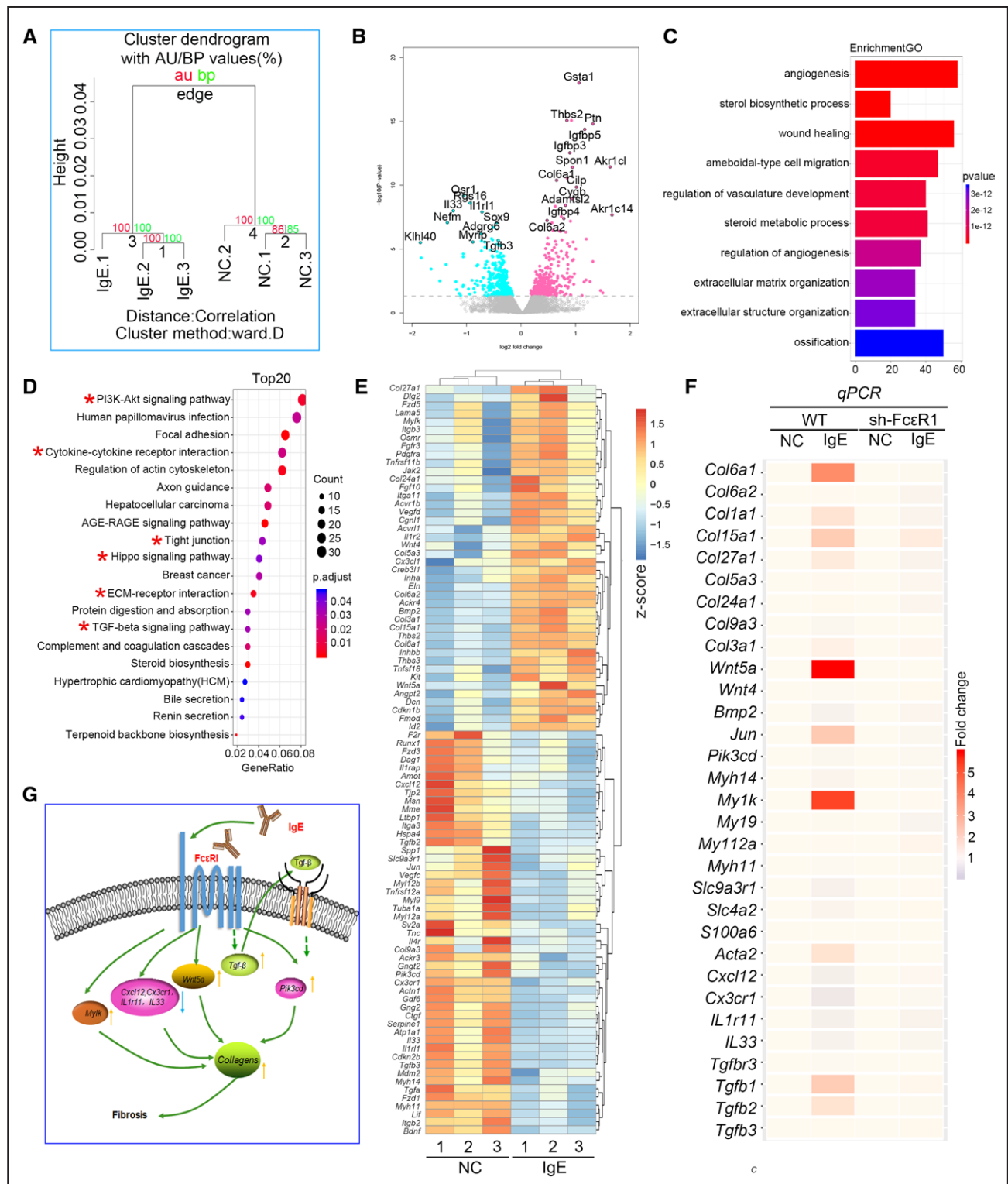


Figure 7. Effects of IgE on transcriptome profile of neonatal rat cardiac fibroblasts.

A, Hierarchical clustering of the RNA sequencing samples on the basis of gene expression levels. **B**, Volcano plot of the differentially expressed genes by *P* value. Pink dots represent genes with increased expression after IgE treatment, and blue dots represent genes with decreased expression. **C**, GO analysis of the differentially expressed genes in response to IgE. **D**, KEGG analysis of potential pathways involved in IgE-induced cardiac fibrosis. **E**, Differentially expressed genes enriched 6 interest pathways shown in **D**, which is marked with the red asterisk. **F**, qPCR analysis of the core factors in KEGG hypertrophy-related pathways in cardiac fibroblasts infected with scramble adenovirus (scramble) or sh-FcεR1 adenovirus (sh-FcεR1) followed by IgE treatment. **G**, Proposed model for the effect of IgE on cardiac fibrosis. AGE-RAGE indicates advanced glycation end products-receptor for AGE; AU, approximately unbiased; BP, bootstrap probability; ECM, extracellular matrix; GO, gene ontology; IgE, immunoglobulin E; KEGG, Kyoto Encyclopedia of Genes and Genomes; NC, negative control; PI3K-AKT, phosphatidylinositol 3 kinase-protein kinase B; qPCR, quantitative PCR; Tgf-β, transforming growth factor-β; ward.D, Ward's minimum variance method; and WT, wild-type.

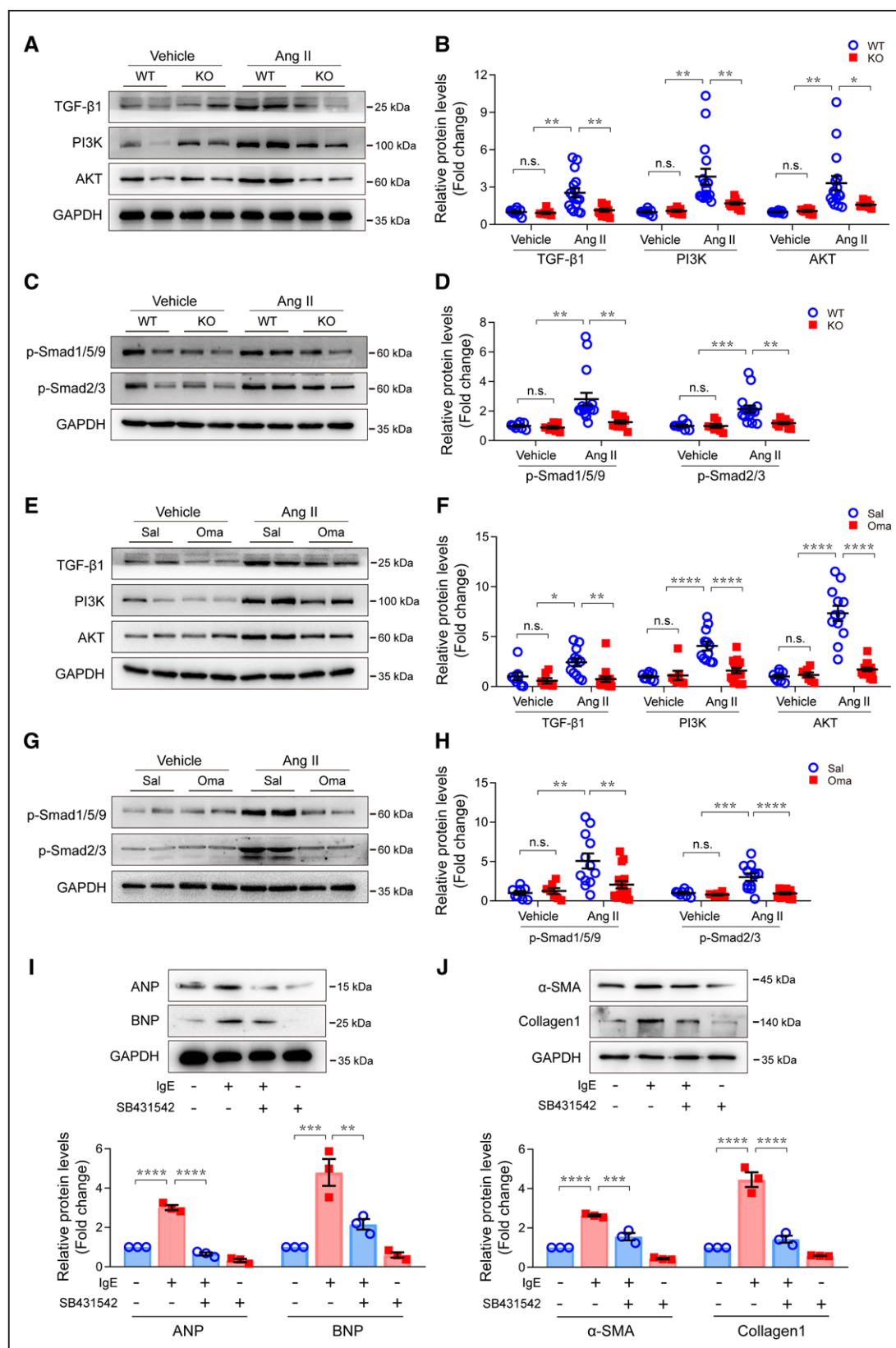


Figure 8. IgE-Fc ϵ R1 induces TGF- β /PI3K-AKT and TGF- β /Smads signal pathways and contributes to the cardiac remodeling.

A, Representative Western blot of TGF- β 1 and downstream molecules (PI3K and AKT) protein expression in WT and Fc ϵ R1-KO heart tissues treated with or without Ang II. **B**, Statistical analysis of TGF- β 1, PI3K, and AKT relative protein expression (fold changes vs Vehicle-WT group). **C**, Representative Western blot of TGF- β downstream factors (pSmad1/5/9 and pSmad2/3) expression in heart tissues of WT and Fc ϵ R1-KO mice with or without Ang II. **D**, Statistical analysis of the pSmad1/5/9 and pSmad2/3 protein expression. **E**, Representative Western blot of TGF- β 1, PI3K, and AKT expression in heart tissues from omalizumab-treated mice with or without Ang II. **F**, Statistical analysis of TGF- β 1, PI3K, and AKT relative protein expression (fold changes vs Vehicle-Saline group). (Continued)

Figure 8 Continued. **G**, Representative western blot of pSmad1/5/9 and pSmad2/3 in heart tissues of omalizumab-treated mice with or without Ang II. **H**, Statistical analysis of the pSmad1/5/9 and pSmad2/3 expression accordingly. **I and J, Top**, Representative Western blot of prohypertrophic markers (ANP and BNP; **I**) and fibrotic markers (α -SMA and collagen 1) protein expression (**J**) in the cardiomyocytes and cardiac fibroblasts after TGF- β inhibitor treatment followed by IgE stimulation. **Bottom**, Statistical analysis of relative protein expression (fold changes vs control group) of prohypertrophic markers (ANP and BNP) and fibrotic markers (α -SMA and collagen 1) in 3 independent experiments. GAPDH was used as the reference control. Total n=8 (vehicle-WT), n=8 (vehicle-KO), n=16 (Ang II-WT), or n=12 (Ang II-KO) per group; n=8 (vehicle-saline), n=7 (vehicle-Oma), n=12 (Ang II-saline) or n=16 (Ang II-Oma) per group. The results are shown as mean $\pm$ SEM of relative fold change compared with reference protein expression. * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001, n.s. indicates no significance in 2-way ANOVA with Bonferroni post hoc test. α -SMA indicates α -smooth muscle actin; AKT, protein kinase B; Ang II, angiotensin II; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; IgE, immunoglobulin E; KO, knockout; Oma, omalizumab; PI3K, phosphatidylinositol 3 kinase; Sal, saline; Tgf- β , transforming growth factor- β ; and WT, wild-type.

VII and XIV in the Data Supplement) and dysfunction in vivo (Table IV in the Data Supplement). More important, we showed the potential therapeutic effects by targeting IgE with IgE-neutralizing antibodies or with the clinical IgE antagonist omalizumab in mice. Thus, our findings suggest that induction of IgE-Fc ϵ R1 may represent a common mechanism associated with pathological cardiac remodeling regardless of allergic reaction, and that IgE-Fc ϵ R1 may represent novel therapeutic targets for cardiac remodeling.

It is generally believed that IgE production requires differentiation of activated IgE(+) B cells into plasma cells in response to stimuli such as allergen sensitization.³⁸ However, we found that in patients with HF with no allergic history, serum IgE levels were also significantly higher than in subjects without HF (Figure 1A). Consistent with these findings, IgE was also shown to be higher in the serum of mice with cardiac remodeling than in control specimens. However, it remains unknown how IgE levels are upregulated in patients and mice with cardiac remodeling. We found that pressure overload led to a marked increase in B cell accumulation in vasculature (data not shown). We thus propose that B cell accumulation in vasculature may result in the production of IgE locally and in circulation, the potential consequences of which warrant further investigation.

IgE is also considered to be a proinflammatory immunoglobulin that functions in immune cells.³⁹ Results from our BM transplantation studies suggest that BM-derived cells, such as macrophages, lymphocytes, mast cells, and neutrophils, do not significantly contribute to IgE-Fc ϵ R1-mediated cardiac remodeling (Figure 4). Our in vitro studies with cultured cardiomyocytes and CFs showed predominant effects of IgE-Fc ϵ R1 in the promotion of cardiomyocyte hypertrophy and CF matrix protein production (Figures 5, 6, and 7). Although these results strongly support the role of IgE-Fc ϵ R1 signaling in cardiomyocytes and CFs, we cannot completely exclude additional contributions from other cardiac resident cells, such as smooth muscle cells, endothelial cells, and pericytes. Among them, resident endothelial cells have been reported to be involved in pathological cardiac remodeling,^{40,41} but a previous study showed that cultured human endothelial cells were insensitive to IgE stimulation.²² In future studies, cell type-specific Fc ϵ R1-KO mice would greatly assist in clarifying the

contributions of individual cardiac cell types as well as noncardiac cells to IgE-Fc ϵ R1-mediated regulation of cardiac remodeling.

There are 2 known IgE receptors, the high-affinity Fc ϵ R1 and the low-affinity Fc ϵ R2.⁴² In this study, we found the upregulation of Fc ϵ R1 in cardiac cells and failing hearts (Figures 1 and 5). Thus, we focused on Fc ϵ R1. Previous studies have suggested that the high- and low-affinity IgE receptors have different expression patterns and functional roles.⁴³ For example, Fc ϵ R2 may contribute to the production of chemokines,⁴² and chemokines have also been shown to function directly in cardiac remodeling.^{44,45} The role of the low-affinity receptor Fc ϵ R2 in IgE-mediated cardiac remodeling/failure remains unclear and deserves to be investigated in the future.

Previous studies have demonstrated that both myocardial and systemic TGF- β , especially TGF- β 1, are increased in patients with cardiac hypertrophy and fibrosis.⁴⁶ In cardiomyocytes and CFs, Ang II induces TGF- β 1 mRNA and protein expression.^{46–48} Treatment with angiotensin-converting enzyme inhibitors or Ang II type I receptor blockers significantly decreases TGF- β 1 expression and alleviates Ang II-induced cardiac remodeling.⁴⁹ These findings suggest TGF- β 1 acts as a downstream mediator of Ang II–Ang II type I receptor signaling. In this study, we found that IgE upregulates TGF- β 1 expression via Fc ϵ R1, suggesting that TGF- β could also be an important mediator of the IgE-Fc ϵ R1 signaling in cardiac hypertrophy and fibrosis. In addition to the TGF- β pathway, our bioinformatic analyses also suggested that the β -adrenergic receptor (β -AR) signaling pathway, chemokine-related pathway, and cytokine-mediated inflammation might also be involved in IgE-mediated cardiac remodeling. In light of reports describing cross-talk and mutual regulation between β -AR and TGF- β signaling,⁵⁰ we propose that β -AR may contribute to TGF- β activation, and regulate cardiac hypertrophy via activation of the cAMP-PKA pathway in cardiomyocytes. The roles of other potential signaling pathways in IgE-Fc ϵ R1-mediated regulation of cardiac remodeling and failure deserve to be investigated in the future. In conclusion, this study extends our understanding the role of IgE-Fc ϵ R1 in pathological cardiac remodeling and failure under different etiologies. Our findings may facilitate the development of novel therapeutic targets for treatment of pathological cardiac remodeling, especially when associated with allergic reactions.

ARTICLE INFORMATION

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H.Z., H.Y., C.G., and Y.C. performed the experiments and analyzed the data. T.S. performed the echocardiogram examination. H.Y., C.H., and C.G. performed experiments in the animal model. Y.T., M.Z., Z.L., B.L., and A.W. blindly statistically analyzed immunostaining images. X.S. and J.P. performed the bioinformatic analysis. X.G. and S.C. provided the human samples and data. B.L. gave valuable comments. J.W. and C.Y. planned the research strategy and revised the article. The authors thank Yanru Wang (Peking University) for rat cardiomyocyte isolation and Dr Xia Wang (Capital University of Medical Sciences) and Dandan Li (National Center for Cardiovascular Diseases) for establishing the TAC-induced cardiac remodeling mouse model.

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Disclosures

None.

Supplemental Materials

Data Supplement Methods
Data Supplement Tables I–X
Data Supplement Figures I–XVI
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