Current Topics

Inflammation and Tissue Remodeling as Potential Therapeutic Targets

Communication to the Editor

IL-1 β Plays an Important Role in Pressure Overload-Induced Atrial Fibrillation in Mice

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Hypertension is one risk for atrial fibrillation (AF) and induces cardiac inflammation. Recent evidence indicates that pressure overload-induced ventricular structural remodeling is associated with the activation of nucleotide binding-oligomerization domain (NOD)-like receptor P3 (NLRP3) inflammasomes, including an apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC). We hypothesized that NLRP3 inflammasomes are an initial sensor for danger signals in pressure overload-induced atrial remodeling, leading to AF. Transverse aortic constriction (TAC) or a sham procedure was performed in mice deficient for ASC^{−/−} and interleukin-1 β (IL-1 β ^{−/−}). One week after the procedure, electrical left atrial burst pacing from the esophagus was performed for 30 s to induce AF. IL-1 β , monocyte chemoattractant protein 1 (MCP-1), connective tissue growth factor (CTGF), and collagen 1 gene expression were also examined. The electrical burst pacing induced AF in TAC-operated wild-type (WT) ($p < 0.001$) and ASC^{−/−} ($p < 0.05$) mice, compared to no AF in the sham-operated WT and ASC^{−/−} mice, respectively. In contrast, the number of mice in which sustained AF was induced was similar between TAC-operated IL-1 β ^{−/−} and sham-operated IL-1 β ^{−/−} mice ($p > 0.05$). The expression of all genes tested was increased in TAC-operated WT and ASC^{−/−} mice compared with sham-operated WT and ASC^{−/−} mouse atria, respectively. CTGF and collagen 1, but not MCP-1, gene expressions were increased in TAC-operated IL-1 β ^{−/−} mouse atria compared with sham-operated WT and IL-1 β ^{−/−} mouse atria. In contrast, the IL-1 β gene was not detected in either TAC-operated or sham-operated IL-1 β ^{−/−} mouse atria. These results suggest that an IL-1 β activation pathway, different from NLRP3 inflammasomes, plays an im-

portant role in pressure overload-induced sustained AF.

Key words NLRP3 inflammasome; hypertension; atrial fibrillation

INTRODUCTION

Atrial fibrillation (AF) is a very common heart rhythm disturbance for which treatment remains problematic. AF is a relatively common complication of hypertension, an important predisposing factor for AF.¹⁾ Therefore, identification of the critical mechanisms involved in hypertension-induced AF is important for the treatment of AF. Some evidence suggests that inflammation plays a significant role in the pathogenesis of AF. For example, some evidence shows the expression of inflammatory markers in cardiac tissues of AF patients²⁾ and a pressure-overloaded mouse model of AF.³⁾ Previous studies have shown that hypertension causes atrial structural remodeling such as atrial fibrosis, dilatation, and ischemia to promote AF.¹⁾ Moreover, left ventricular pressure-overload can induce atrial stretching, suggesting that it induces atrial inflammation.⁴⁾ Recently, chronic pressure overload-induced ventricular structural remodeling induced by transverse aortic constriction (TAC), an animal model of hypertension, has been associated with the activation of nucleotide binding-oligomerization domain and leucine-rich repeat containing protein 3 (NLRP3) inflammasomes including an apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC).⁵⁾ However, whether the activation of NLRP3 inflammasome participates in the hypertension-induced atrial remodeling and AF has remained unclear. We examined the role of NLRP3 inflammasome in the development of hypertension-induced AF using TAC-operated ASC-deficient (ASC^{−/−}) and interleukin 1 β -deficient (IL-1 β ^{−/−}) mice.

MATERIALS AND METHODS

Ethics This study was conducted in strict accordance with the recommendations in the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. This study was approved by the Animal Care Committee of Iwate Medical University. The protocol was approved by the Committee on the Ethics of Animal Experiments of Iwate Medical University (Permit Number: 24–002).

Experimental Animals and TAC Operation ASC^{−/−} and IL-1 β ^{−/−} mice were kindly provided by Dr. S. Taniguchi (Shinshu University, Matsumoto, Japan)⁶⁾ and Dr. Yoichiro Iwakura (Tokyo University of Science, Chiba, Japan),⁷⁾ respectively. C57BL/6J wild-type (WT) mice were purchased from Japan SLC, Inc. (Tokyo, Japan). To create pressure overload, thoracic TAC was performed as described elsewhere.⁸⁾ Briefly, all mice (9-weeks old) were anesthetized with isoflurane (2–4%) and intubated with a 22-gauge polyethylene catheter.

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After mice were ventilated with a rodent ventilator (Harvard Apparatus, Holliston, MA, U.S.A.), the chest was opened at the left second intercostal space and the transverse section of the aorta was freed. A 7-0 nylon suture was passed around the aorta between the right innominate and left common carotid arteries. The aorta was tied with a nylon suture using a 27-gauge needle, and the needle was promptly removed. In the sham-operated mice, the same procedure was performed except for the ligation. Finally, the chest wall was closed with negative pressure in the chest cavity. One week after surgery, all experiments were performed on all types of mice. Sodium pentobarbital (30 mg/kg) applied intraperitoneally, and isoflurane inhalation, were used to anesthetize all animals. The adequacy of anesthesia was monitored by heart rate, the degree of motion of the sternum, and movement of the extremities.

Experimental Protocol for Atrial Fibrillation Induction One week after TAC operation, two platinum bipolar electrodes with 1 mm interelectrode spacing were inserted into the esophagus to stimulate the epicardial surface of the left atrium and to record the atrial electrogram, respectively. The electrocardiogram (ECG) lead II was also recorded, and each of 2 electrographic signals was filtered (1.0–1000 Hz), digitized to 16-bit precision at a sample rate of 1000 Hz per channel (PowerLab AD Instruments, Pty. Ltd., Bella Vista, New South Wales, Australia), transmitted to a microcomputer, and saved to a hard disk drive. The remaining bipolar electrode was connected to an electrical stimulator (SEN7013, Nihon Kohden, Tokyo, Japan). One week after TAC operation, electrical left atrial burst pacing ($S_1S_1 = 20$ ms) for thirty seconds in the esophagus was performed to induce AF. Sustained AF was defined as an irregular atrial rhythm (atrial CL less than 80 ms) persisting longer than 5 min. When burst pacing induced AF, the AF terminated spontaneously within 5 min. AF was reinitiated at the same cycle length. Each experiment ended after burst pacing was performed 5 times, or when burst pacing induced AF lasting more than 5 min.

Quantification of mRNA by Real-Time PCR Total RNA was prepared from the atrial myocardium of anesthetized sham-operated WT, ASC^{-/-}, and IL-1 β ^{-/-} mice and TAC-operated WT, ASC^{-/-}, and IL-1 β ^{-/-} mice ($n = 8$ for each) using the ReliaPrepTM RNA Tissue Miniprep System (Promega, Madison, WI, U.S.A.) according to the manufacturer's instructions. Five hundred nanograms of total RNA was used as a template for reverse transcription using the SuperScript[®] III First-Strand synthesis system (Invitrogen, Carlsbad, CA, U.S.A.). Real-time RT-PCR analysis was performed with an ABI Step One Real-Time PCR System using the Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA, U.S.A.) to detect IL-1 β , monocyte chemotactic protein 1 (MCP-1), connective tissue growth factor (CTGF), collagen type I (collagen 1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The expression of each gene was normalized to that of GAPDH mRNA because the expression of GAPDH mRNA was constant between groups.

Data Analysis All data are shown as the mean $\pm$ standard error (S.E.). An ANOVA by Bonferroni's test was used for statistical analysis of multiple comparisons of data. $p < 0.05$ was considered significant.

RESULTS

Sustained Atrial Fibrillation (AF) and ECG Parameters

To evaluate whether NLRP3 inflammasomes may participate in the induction of sustained AF in pressure overloaded mouse atria, the left atrial electrical burst pacing in the esophagus was performed in TAC-operated WT, ASC^{-/-}, and IL-1 β ^{-/-} mice. A representative AF induction is shown in Fig. 1. The left atrial burst pacing induced AF in a TAC-operated ASC^{-/-} mouse at one week after TAC operation. A surface electrocardiogram showed a rapid rhythm with irregular R–R intervals, indicating AF. The frequency of incidence of sustained AF induction is shown on Table 1. The electrical burst pacing induced AF for more than 5 min (*i.e.*, sustained AF) in TAC-operated WT mice compared with the sham-operated WT (*i.e.*, Control) mice ($p < 0.001$). The burst pacing also induced sustained AF in TAC-operated ASC^{-/-} mice, greater than that in sham-operated ASC^{-/-} mice ($p < 0.05$). In contrast, the number of mice in which sustained AF was induced was similar between TAC-operated IL-1 β ^{-/-} and sham-operated IL-1 β ^{-/-} mice ($p > 0.05$). ECG parameters are shown in Table 2. All ECG parameters were similar among six different groups of mice.

Gene Expression Levels of IL-1 β , MCP-1, CTGF and Collagen 1 We examined the effects of pressure overload on atrial mRNA expression of IL-1 β , MCP-1, CTGF and collagen 1 in six different groups of mouse hearts (sham-operated

Fig. 1. Example of Atrial Fibrillation (AF) Induced by Electrical Left Atrial Burst Pacing from the Esophagus in a TAC-Operated ASC^{-/-} Mouse

Representative example of atrial fibrillation (AF) induced by the burst pacing at a cycle length of 20 ms for 30 s lasting more than 5 min in a TAC-operated ASC^{-/-} mouse at 10 weeks old. (Color figure can be accessed in the online version.)

Table 1. The Frequency of Incidence of Sustained Atrial Fibrillation (SAF) in Six Different Groups of Mice

Parameters	Total number of mice	SAF (–)	SAF (+)	<i>p</i> Value
WT (TAC–)	30	27	3	<0.01
WT (TAC+)	30	15	15	
ASC ^{-/-} (TAC–)	30	25	5	<0.05
ASC ^{-/-} (TAC+)	30	16	14	
IL-1 β ^{-/-} (TAC–)	30	24	6	NS
IL-1 β ^{-/-} (TAC+)	30	24	6	

Statistical analysis was performed using the chi-square test (Fisher's exact test). TAC+, transverse aortic constriction operated; TAC–, sham operated; WT, wild-type mouse; ASC-KO, mice deficient for apoptosis-associated speck-like adaptor protein; IL-1 β -KO, mice deficient for interleukin-1 β . NS, Not significant.

and TAC-operated WT, $ASC^{-/-}$, and $IL-1\beta^{-/-}$ mouse hearts). $IL-1\beta$ gene expression was increased in TAC-operated WT and $ASC^{-/-}$ mouse atria compared with the sham-operated WT and $ASC^{-/-}$ mouse atria, respectively (Fig. 2A). In contrast, $IL-1\beta$ gene expression was not detected in either the TAC-operated or sham-operated $IL-1\beta^{-/-}$ mouse atria. Interestingly, MCP-1 gene expression was significantly increased in TAC-operated WT and $ASC^{-/-}$ mouse atria, but not in $IL-1\beta^{-/-}$ mouse atria, compared with the sham-operated WT mouse atria (Fig. 2B). The gene expression of both CTGF and collagen 1 was significantly upregulated in TAC-operated WT, $ASC^{-/-}$, and $IL-1\beta^{-/-}$ mouse atria compared with that of sham-operated WT mouse atria, (Figs. 2C–D). Moreover, the gene expression was significantly upregulated in TAC-operated $ASC^{-/-}$, and $IL-1\beta^{-/-}$ mouse atria compared with that of sham-operated $ASC^{-/-}$, and $IL-1\beta^{-/-}$ mouse atria, respectively.

DISCUSSION

Our present study demonstrates that TAC (*i.e.*, pressure

overload) significantly increases the frequency of the induction of sustained AF in WT and $ASC^{-/-}$, but not in $IL-1\beta^{-/-}$, mice. Moreover, TAC increased gene expression levels of $IL-1\beta$ in WT and $ASC^{-/-}$, but not in $IL-1\beta^{-/-}$, mice. Previous studies have also demonstrated that pressure overload increases mRNA expression of $IL-1\beta$ in the heart.^{9,10} These results suggest that $IL-1\beta$ plays an important role in the development of sustained AF induced by pressure overload. A recent study has demonstrated that chronic pressure overload-induced ventricular structural remodeling induced by TAC is associated with the activation of NLRP3 inflammasomes, which includes the adapter protein ASC.⁵ However, NLRP3 inflammasome activation induces caspase 1 activation, leading to processing pro- $IL-1\beta$ into its active form $IL-1\beta$. Therefore, the present results suggest that NLRP3 inflammasomes might not participate in the development of sustained AF in a mouse model of pressure overload. It is known that the processing of pro- $IL-1\beta$ into its active $IL-1\beta$ is also mediated by neutrophil-derived serine proteases, or by proteases delivered by invading microorganisms, independent from NLRP3 inflammasomes.^{11,12} It is known that the major cellular sources of $IL-1\beta$ are monocytes, macrophages, and the endothelium, and that pressure overload can markedly increase the density of macrophages 7d after TAC in mouse hearts.¹³ However, the pressure overload moderately increased the density of neutrophils 3d after TAC, but this did not reach a statistically significant level in mouse hearts.¹³ Moreover, we did not elucidate the release of neutrophil-derived serine proteases in this mouse model of pressure overload. Therefore, the mechanism for the processing pro- $IL-1\beta$ into its active $IL-1\beta$ remains unclear.

It is well known that $IL-1\beta$ induces MCP-1 gene expression, and conversely, that its gene expression is also induced

Table 2. Electrocardiogram Parameters

Parameters	RR (ms)	P (ms)	PQ (ms)	QRS (ms)	QT (ms)
WT (TAC-)	136 ± 12	8 ± 0.3	41 ± 1.6	9 ± 1	34 ± 1
WT (TAC+)	127 ± 8	9 ± 0.9	40 ± 2.1	10 ± 1	34 ± 1
$ASC^{-/-}$ (TAC-)	143 ± 13	11 ± 0.2	44 ± 1.8	10 ± 1	34 ± 1
$ASC^{-/-}$ (TAC+)	129 ± 2	10 ± 0.5	45 ± 1.7	11 ± 1	36 ± 1
$IL-1\beta^{-/-}$ (TAC-)	142 ± 11	8 ± 1.4	40 ± 1.7	11 ± 3	31 ± 1
$IL-1\beta^{-/-}$ (TAC+)	121 ± 5	8 ± 0.6	39 ± 2.5	11 ± 1	34 ± 3

Each value represents the mean ± S.E.

Fig. 2. Quantitative Analyses of Interleukin 1β ($IL-1\beta$) (Panel A), Monocyte Chemotactic Protein 1 (MCP-1) (Panel B), Connective Tissue Growth Factor (CTGF) (Panel C), and Collagen Type 1 (Collagen 1) (Panel D) Gene Expression by Real-Time RT-PCR in Six Different Groups of Mouse Atria at 10 Weeks Old

Data for $IL-1\beta$, MCP-1, CTGF, and collagen 1 were normalized to those for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data are the mean ± S.E. obtained from 6 mice for each group. $p < 0.05$ was considered significant. Sham-operated, TAC (-); TAC-operated, TAC (+).

by MCP-1, suggesting that the induction of each other would amplify the biological effects of these cytokines during pressure overload.^{14,15)} Atrial remodeling is associated with pressure overload-induced increases in MCP-1 expression, leading to AF.^{16,17)} Atrial interstitial fibrosis as an atrial remodeling is associated with increases in CTGF and collagen 1 gene expression. Our results demonstrated that pressure overload increased the gene expression of both IL-1 β and MCP-1, leading to sustained AF (Fig. 2). However, the increased CTGF and collagen 1 gene expression did not increase the number of mice in which AF was induced among TAC-operated IL-1 $\beta^{-/-}$ mice. These results suggest that IL-1 β and MCP-1 are more important than CTGF and collagen 1 in the induction of sustained AF during the relatively short period of pressure overload.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

- Ogunsua AA, Shaikh AY, Ahmed M, McManus DD. Atrial Fibrillation and Hypertension: Mechanistic, Epidemiologic, and Treatment Parallels. *Methodist Debakey Cardiovasc. J.*, **11**, 228–234 (2015).
- Yamashita T, Sekiguchi A, Iwasaki YK, Date T, Sagara K, Tanabe H, Suma H, Sawada H, Aizawa T. Recruitment of immune cells across atrial endocardium in human atrial fibrillation. *Circ. J.*, **74**, 262–270 (2010).
- Liao C, Akazawa H, Tamagawa M, Ito K, Yasuda N, Kudo Y, Yamamoto R, Ozasa Y, Fujimoto M, Wang P, Nakauchi H, Nakaya H, Komuro I. Cardiac mast cells cause atrial fibrillation through PDGF-A-mediated fibrosis in pressure-overloaded mouse hearts. *J. Clin. Invest.*, **120**, 242–253 (2010).
- Oishi S, Sasano T, Tateishi Y, Tamura N, Isobe M, Furukawa T. Stretch of atrial myocytes stimulates recruitment of macrophages via ATP released through gap-junction channels. *J. Pharmacol. Sci.*, **120**, 296–304 (2012).
- Li R, Lu K, Wang Y, Chen M, Zhang F, Shen H, Yao D, Gong K, Zhang Z. Triptolide attenuates pressure overload-induced myocardial remodeling in mice via the inhibition of NLRP3 inflammasome expression. *Biochem. Biophys. Res. Commun.*, **485**, 69–75 (2017).
- Yamamoto M, Yaginuma K, Tsutsui H, Sagara J, Guan X, Seki E, Yasuda K, Yamamoto M, Akira S, Nakanishi K, Noda T, Taniguchi S. ASC is essential for LPS-induced activation of procaspase-1, independently of TLR-associated signal adaptor molecules. *Genes Cells*, **9**, 1055–1067 (2004).
- Horai R, Asano M, Sudo K, Kanuka H, Suzuki M, Nishihara M, Takahashi M, Iwakura Y. Production of mice deficient in genes for interleukin (IL)-1 α , IL-1 β , IL-1 α/β , and IL-1 receptor antagonists shows that IL-1 β is crucial in turpentine-induced fever development and glucocorticoid secretion. *J. Exp. Med.*, **187**, 1463–1475 (1998).
- Niizeki T, Takeishi Y, Kitahara T, Arimoto T, Ishino M, Bilim O, Suzuki S, Sasaki T, Nakajima O, Walsh RA, Goto K, Kubota I. Diacylglycerol kinase-epsilon restores cardiac dysfunction under chronic pressure overload: a new specific regulator of Galpha(q) signaling cascade. *Am. J. Physiol. Heart Circ. Physiol.*, **295**, H245–H255 (2008).
- Shioi T, Matsumori A, Kihara Y, Inoko M, Ono K, Iwanaga Y, Yamada T, Iwasaki A, Matsushima K, Sasayama S. Increased expression of interleukin-1 beta and monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 in the hypertrophied failing heart with pressure overload. *Circ. Res.*, **81**, 664–671 (1997).
- Mao Y, Tokudome T, Kishimoto I, Otani K, Nishimura H, Yamaguchi O, Otsu K, Miyazato M, Kangawa K. Endogenous ghrelin attenuates pressure overload-induced cardiac hypertrophy via a cholinergic anti-inflammatory pathway. *Hypertension*, **65**, 1238–1244 (2015).
- Dinarello CA, Bendtzen K, Wolff SM. Studies on the active site of human leukocytic pyrogen. *Inflammation*, **6**, 63–78 (1982).
- Netea MG, Simon A, van de Veerdonk F, Kullberg BJ, Van der Meer JWM, Joosten LAB. IL-1 β processing in host defense: beyond the inflammasomes. *PLoS Pathog.*, **6**, e1000661 (2010).
- Xia Y, Lee K, Li N, Corbett D, Mendoza L, Frangogiannis NG. Characterization of the inflammatory and fibrotic response in a mouse model of cardiac pressure overload. *Histochem. Cell Biol.*, **131**, 471–481 (2009).
- Parry GC, Martin T, Felts KA, Cobb RR. IL-1 β -induced monocyte chemoattractant protein-1 gene expression in endothelial cells is blocked by proteasome inhibitors. *Arterioscler. Thromb. Vasc. Biol.*, **18**, 934–940 (1998).
- Gavrilin MA, Deucher MF, Boeckman F, Kolattukudy PE. Monocyte chemotactic protein 1 upregulates IL-1 β expression in human monocytes. *Biochem. Biophys. Res. Commun.*, **277**, 37–42 (2000).
- Kume O, Teshima Y, Abe I, Ikebe Y, Oniki T, Kondo H, Saito S, Fukui A, Yufu K, Miura M, Shimada T, Takahashi N. Role of atrial endothelial cells in the development of atrial fibrosis and fibrillation in response to pressure overload. *Cardiovasc. Pathol.*, **27**, 18–25 (2017).
- Kume O, Takahashi N, Wakisaka O, Nagano-Torigoe Y, Teshima Y, Nakagawa M, Yufu K, Hara M, Saikawa T, Yoshimatsu H. Pioglitazone attenuates inflammatory atrial fibrosis and vulnerability to atrial fibrillation induced by pressure overload in rats. *Heart Rhythm*, **8**, 278–285 (2011).