Atherosclerosis 289 (2019) 14–20

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Deletion of interleukin-18 attenuates abdominal aortic aneurysm formation

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HIGHLIGHTS

- IL-18 deletion attenuated abdominal aortic aneurysm (AAA) formation.
- IL-18 induced enhanced expression of osteopontin in vascular smooth muscle cells.
- IL-18 contributes to inflammation and matrix metalloproteinase activation in AAA.

ARTICLE INFO

Keywords:
Abdominal aortic aneurysm
Interleukin-18
Macrophage
Osteopontin
Matrix metalloproteinase

ABSTRACT

Background and aims: Abdominal aortic aneurysm (AAA) is a common disease; however, its exact pathogenesis remains unknown, and no specific medical therapies are available. Interleukin (IL)-18 plays a crucial role in atherosclerotic plaque destabilization and is a strong predictor of cardiovascular death. Here, we investigated the role of IL-18 in AAA pathogenesis using an experimental mouse model.

Methods and results: After infusion of angiotensin II (Ang II) for 4 weeks and β-aminopropionitrile (BAPN) for 2 weeks, 58% of C57/6J wild-type (WT) mice developed AAA associated with enhanced expression of IL-18; however, disease incidence was significantly lower in *IL-18*$^{-/-}$ mice than in WT mice ($p < 0.01$), although no significant difference was found in systolic blood pressure between WT mice and *IL-18*$^{-/-}$ mice in this model. Additionally, IL-18 deletion significantly attenuated Ang II/BAPN-induced macrophage infiltration, macrophage polarization into inflammatory M1 phenotype, and matrix metalloproteinase (MMP) activation in abdominal aortas, which is associated with reduced expression of osteopontin (OPN).

Conclusions: These findings indicate that IL-18 plays an important role in the development of AAA by enhancing OPN expression, macrophage recruitment, and MMP activation. Moreover, IL-18 represents a previously unrecognized therapeutic target for the prevention of AAA formation.

1. Introduction

Abdominal aortic aneurysm (AAA) is a common aortic disease defined as a focal dilatation of the aortic diameter > 1.5-times the normal segment. Previous ultrasound-screening studies showed that the prevalence of AAA in individuals > 55 years of age ranges from 4% to 7% in males and 1%–2% in females [1–3]. Many patients with AAA remain completely asymptomatic; however, AAA rupture can still occur in some patients and is nearly uniformly fatal [4]. Surgical interventions are only an option for patients when the aneurysm diameter reaches ≥5.5 cm, and optimal medical therapy to control AAA expansion has not been developed yet [5,6]; therefore, elucidating the mechanism involved in AAA initiation and progression is increasingly required to discover new treatment strategies for this disease.

The pathological features of AAA are characterized by infiltration of inflammatory cells, such as macrophages, neutrophils, mast cells, plasmacytoid dendritic cells, $CD4^+$ T cells, and B cells, extracellular-matrix (ECM) degradation, and reduction of vascular smooth muscle cells (VCMCs) [7–11]. Although the cellular and molecular mechanisms have not been entirely determined, many recent studies provide

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https://doi.org/10.1016/j.atherosclerosis.2019.08.003
Received 15 November 2018; Received in revised form 4 July 2019; Accepted 16 August 2019
Available online 17 August 2019
0021-9150/

important insights into AAA pathogenesis [5,12]. The factors associated with inflammation include epidermal growth factor, interleukin (IL)-1β, and IL-17, which regulate leukocyte recruitment [13]. Additionally, osteopontin (OPN), a matrix protein identified as a potential to bridge between cells and hydroxyapatite, is another regulator of inflammatory cell recruitment and adhesion [14]. Two major classes of proteases are involved in ECM degradation: matrix metalloproteinases (MMPs) and cathepsins. Among these, MMP-1, -2, -3, -9, -13, and −14 and cathepsin G, K, L, and S are commonly associated with AAA pathogenesis [11,15]. These proteases are synthesized and secreted by various cells, including macrophages, neutrophils, and smooth muscle cells. Accumulating evidence indicates that mast-cell-derived proteases (e.g., cathepsin G, chymase, and tryptase) induce activation of MMPs and apoptosis of VSMCs, thereby contributing to AAA formation [9]. Furthermore, OPN induces MMP production [16] and contributes to aneurysmal formation in mouse models [17,18].

IL-18 is a member of the IL-1 cytokine family and expressed by both immune and non-immune cells to contribute to the pathophysiology of various diseases [19]. IL-18 reportedly regulates OPN production in several cell types, including cardiac fibroblasts and peripheral blood mononuclear cells [17,20]. In atherosclerosis, IL-18 promotes plaque progression and plaque instability [21]. However, the precise role of IL-18 in AAA development remains elusive.

In this study, we investigated the role of IL-18 in AAA pathogenesis using an experimental mouse model in order to test the hypothesis that IL-18 plays an important role in AAA formation.

2. Materials and methods

See Supplementary Materials and methods for additional details.

2.1. Animals and treatment

All animal procedures were approved by the Animal Studies Committee of Ehime University. Eight-week-old male C57BL/6J wild-type (WT) mice were purchased from Charles River Laboratories Japan Inc. (Atsugi, Japan), and *Il-18*$^{-/-}$ mice with the same C57BL/6J genetic background were kindly provided by Professor Hideki Okamura (Hyogo College of Medicine, Nishinomiya, Japan). Standard commercial diet and water were available ad libitum. The mice were infused with vehicle or angiotensin II (Ang II; 1000 ng/kg/min; Sigma-Aldrich, Tokyo, Japan) for 4 weeks through an osmotic minipump (Alzet model 2004; Durect, Cupertino, CA, USA), which was implanted subcutaneously. All animals were also treated with β-aminopropionitrile (BAPN; 1 mg/mL), a lysyl oxidase inhibitor, via oral administration for the first 2 weeks [22,23]. Mice were anesthetized during surgery with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Health status of the mice was regularly monitored by the animal caretakers during the study. Mice that died from unknown cause within 24 h of surgery were excluded from the study. At 28 days after the osmotic minipump implantation, mice were sacrificed, and the aorta was used for experimental studies. The maximum abdominal aortic diameter was measured adventitia to adventitia to determine the largest external diameter of the abdominal aorta in phosphate-buffered saline without any physical stretching by using a digital caliper (Hunan Andrew International Industry & Trading Co., Ltd., Hunan, China) under a dissection microscope.

2.2. Statistical analysis

All data are expressed as the mean ± standard error of the mean. Analysis between two groups was performed with the Mann-Whitney *U* test, and differences among more than two groups were tested by analysis of variance, followed by the Tukey post-hoc test. Statistic relationships between different parameters were evaluated using Spearman's rank correlation coefficient. Analyses were performed using SPSS software (V.22.0; IBM Japan, Ltd., Tokyo, Japan, and a $p < 0.05$ was considered statistically significant.

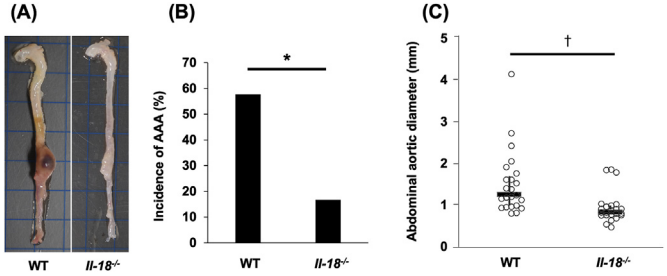

Fig. 1. Interleukin (IL)-18 deletion attenuates the development of abdominal aortic aneurysm (AAA).
(A) Representative images of the whole aorta from the angiotensin II (Ang II) plus β-aminopropionitrile (BAPN)-induced aneurysm model wild-type (WT) and *Il-18*$^{-/-}$ mice. (B) Incidence of AAA (n = 26–29 per group). (C) Abdominal aortic diameter was measured on the suprarenal abdominal aorta (n = 26–29 per group). All values are presented as the mean ± standard error of the mean. *$p < 0.01$; †$p < 0.001$. Data were analyzed using Mann-Whitney *U* test.

3. Results

3.1. IL-18 deletion attenuates Ang II/BAPN-induced AAA formation in vivo

To elucidate the effects of IL-18 on AAA formation, we initially evaluated the diameter of the abdominal aorta in Ang II/BAPN-induced WT and *Il-18*$^{-/-}$ mouse model of AAA. After infusion of Ang II for 4 weeks and BAPN for 2 weeks, 58% of WT mice developed AAA associated with enhanced expression of *Il-18* (Fig. 1 and Supplementary Fig. 1), with AAA incidence significantly lower in *Il-18*$^{-/-}$ mice than in WT mice (Fig. 1). Additionally, enhanced *Il-18* expression in abdominal aortic sections of Ang II/BAPN-infused WT mice was colocalized with α-smooth muscle actin (α-SMA)-positive cells (Supplementary Fig. 1). This result indicated that the main source of IL-18 in this AAA mouse model was VSMCs. Maximal diameter of the abdominal aorta was significantly decreased in *Il-18*$^{-/-}$ mice as compared with that in WT mice (Fig. 1). As shown in Supplementary Fig. 2, 15% of WT mice infused with Ang II/BAPN died from aortic rupture, whereas only 4% of *Il-18*$^{-/-}$ mice died. Furthermore, no significant difference was found in the systolic blood pressure and body weight between WT mice and *Il-18*$^{-/-}$ mice in this model (Supplementary Fig. 3). These results suggested that IL-18 played an important role in AAA formation induced by Ang II and BAPN. Because IL-1β and IL-17 have also been associated with the development of AAA [24,25], we examined *Il-1β* and *Il*-17 mRNA levels in treated WT and *Il-18*$^{-/-}$ mice, finding no significant difference in levels of each mRNA in abdominal aortic tissues between Ang II/BAPN-infused WT and *Il-18*$^{-/-}$ mice (Supplementary Figs. 4A and B).

3.2. IL-18 deletion attenuates macrophage infiltration in abdominal aortic tissues

Infiltration of macrophages is one of the most important events in the development and progression of AAA [7]. To investigate the effect of IL-18 on macrophage infiltration, we performed immunohistochemical staining of F4/80 in abdominal aortic sections. Abundant accumulation of F4/80-positive macrophages was observed in abdominal aortic sections of Ang II/BAPN-infused WT mice but not in *Il-18*$^{-/-}$ mice (Fig. 2A). Flow cytometric analysis revealed that the number of CD68-positive macrophages infiltrating into abdominal aortic tissues in *Il-18*$^{-/-}$ mice was significantly smaller than that in WT mice (Fig. 2B). Moreover, the maximum abdominal aortic diameter was positively associated with *Il*-18 mRNA levels and CD68^{+} cell numbers

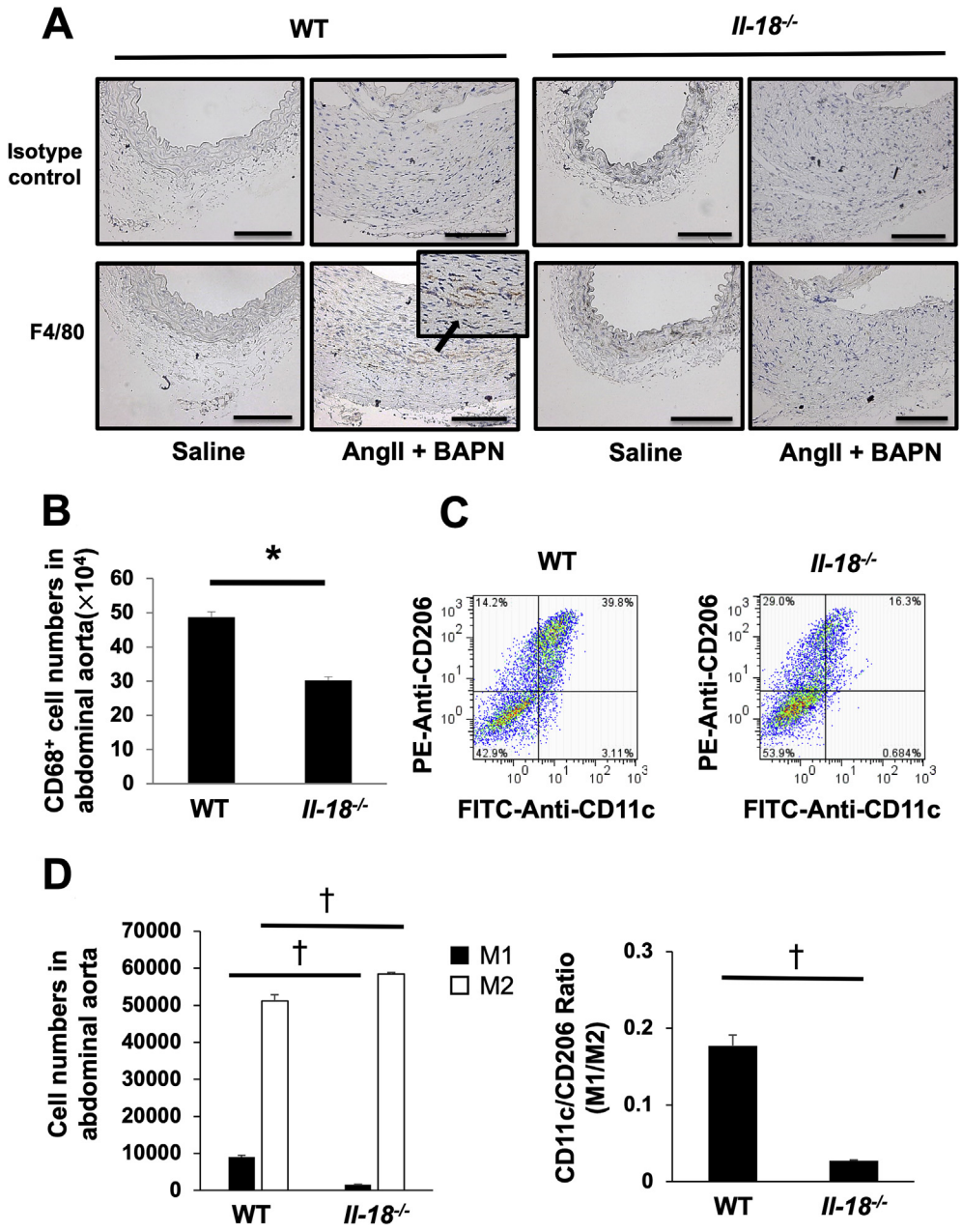

Fig. 2. Interleukin (IL)-18 deletion attenuates macrophage infiltration and polarization into the inflammatory M1 phenotype. (A) Representative images of immunohistochemical staining for F4/80-positive macrophages in wild-type (WT) and *Il-18*$^{-/-}$ abdominal aortas after saline or angiotensin II (Ang II) plus β-aminopropionitrile (BAPN) infusion. Scale bar, 100 μm. (B) Quantification of CD68-positive macrophages by flow cytometry in abdominal aortas from WT or *Il-18*$^{-/-}$ mice after Ang II plus BAPN infusion (n = 5 per group). (C) Representative images of flow cytometric analysis for CD11c^{+}CD68^{+} (M1) or CD206^{+}CD68^{+} (M2) macrophages in abdominal aortas from WT and *Il-18*$^{-/-}$ mice after Ang II plus BAPN infusion. (D) Quantification of M1 and M2 macrophages by flow cytometry in abdominal aortas from WT and *Il-18*$^{-/-}$ after Ang II plus BAPN infusion (n = 5 per group). All values are presented as the mean ± standard error of the mean. *$p < 0.001$; †$p < 0.01$. Data were analyzed using Mann-Whitney *U* test.

in abdominal aortas (Supplementary Fig. 5). These results indicate that IL-18 contributes to macrophage infiltration in abdominal aortic tissues during development and progression of AAA. There was no significant difference in mRNA levels of cell adhesion molecules, including *vascular cell adhesion molecule-1*, *intracellular adhesion molecule-1*, and *P-selectin*, in abdominal aortic tissues between Ang II/BAPN-infused WT and *Il-18*$^{-/-}$ mice (Supplementary Fig. 4C–E). Because impaired macrophage cholesterol efflux has been postulated to play a role in AAA pathogenesis [26], we examined mRNA levels of *scavenger receptor-A*, *CD36*, and *ATP-binding cassette transporter A-1*, finding no statistically significant difference in these levels in abdominal aortic tissues between Ang II/BAPN-infused WT and *Il-18*$^{-/-}$ mice (Supplementary Fig. 6).

3.3. IL-18 deletion affects macrophage polarization

We then examined the M1/M2 polarization of macrophages infiltrating into abdominal aortic tissues to further explore the possible role of IL-18 in macrophage function in AAA. Polarization was assessed using flow cytometric analysis of CD68^{+}CD11c^{+}CD206^{-} or CD68^{+}CD11c^{-}CD206^{+} macrophages. We found that IL-18 deficiency promoted macrophage polarization toward the anti-inflammatory (M2) phenotype rather than the proinflammatory (M1) phenotype (Fig. 2C and D), with M1:M2 ratio significantly lower in the abdominal aortas of *Il-18*$^{-/-}$ mice than that in WT mice (Fig. 2D).

3.4. IL-18 deletion attenuates MMP activation and OPN expression in AAA

We then examined MMP activity in the abdominal aortic tissues of WT and *Il-18*$^{-/-}$ mice. Evaluation of levels of active and proform MMP-2 and MMP-9 using gelatin zymography in the abdominal aortic tissues of Ang II/BAPN-infused *Il-18*$^{-/-}$ mice revealed significant decrease as compared with those in Ang II/BAPN-infused WT mice (Fig. 3). IL-18 reportedly induces the expression of various chemokines, including OPN, in cardiac fibroblast [17], and OPN reportedly contributes to AAA pathogenesis, at least in part, by inducing the MMP

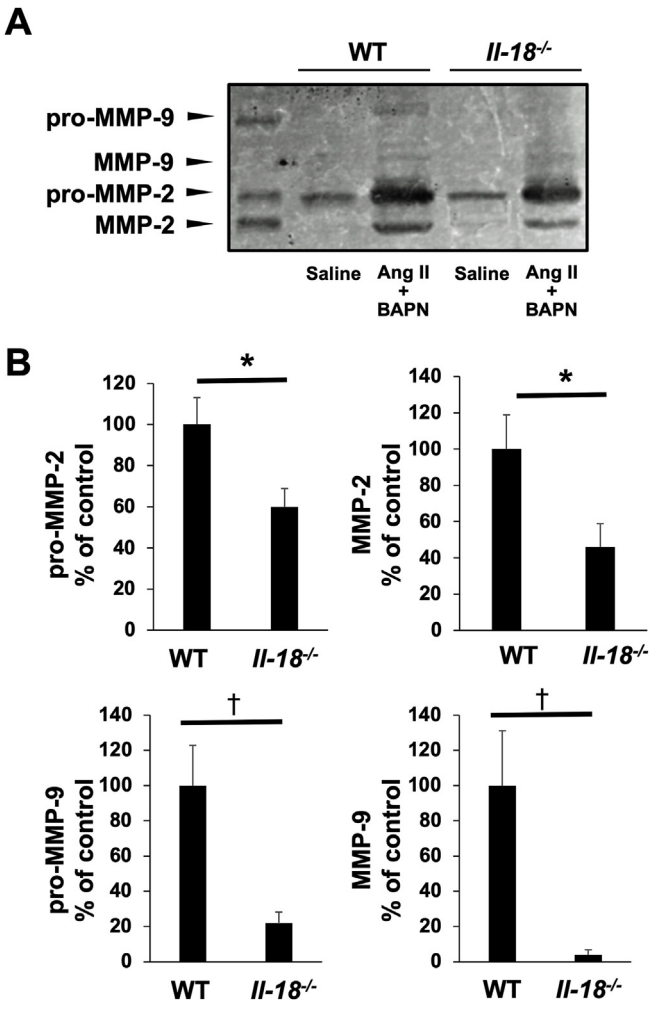

Fig. 3. Interleukin (IL)-18 deletion attenuates matrix metalloproteinase (MMP) activity in the abdominal aorta.
(A) Representative gelatin zymogram of levels of active form and proform of MMP-2 and MMP-9. Protein extracts from the abdominal aorta after saline or angiotensin II plus β-aminopropionitrile infusion in wild-type (WT) and *Il-18*$^{-/-}$ mice were analyzed. (B) Densitometric analysis of changes in MMP activity in treated mice relative to that in control (saline-infused) mice (n = 4 per group). All values are presented as the mean ± standard error of the mean. $^{*}p < 0.05$; $^{\dagger}p < 0.01$. Data were analyzed using Mann-Whitney *U* test.

expression [18]. Therefore, we evaluated the expression of Opn in abdominal aortic tissues. Immunohistochemical staining revealed that Opn was highly expressed in the abdominal aortic sections of Ang II/BAPN-infused WT mice, whereas these levels were markedly decreased in *Il-18*$^{-/-}$ mice (Fig. 4A). Moreover, Opn expression in the abdominal aortic sections of WT mice generally colocalized with α-SMA-positive cells, indicating that Opn is expressed mainly in VSMCs (Fig. 4A). Furthermore, *Opn* mRNA levels in abdominal aortic tissues were significantly decreased in *IL-18*$^{-/-}$ mice relative to WT mice (Fig. 4B). To confirm the source of *Opn* expression, we examined the influence of IL-18 using rat aortic smooth muscle cells, finding that IL-18 stimulation significantly enhanced *Opn* mRNA levels, with maximal values observed at 1 h post-stimulation (Fig. 4C).

3.5. OPN induces macrophage migration

Macrophage infiltration and subsequent inflammation of the vessel wall are speculated to be important contributors to the pathogenesis of aortic aneurysm. Cell migration assays using resident mouse peritoneal macrophages revealed that OPN significantly induced macrophage migration in a dose-dependent manner (Fig. 5A and B) but had no obvious effect on macrophage proliferation (Fig. 5C). Taken together, our results indicated that deletion of IL-18 attenuated AAA formation by inhibiting OPN-induced inflammation and MMP activation.

4. Discussion

Many mouse models that are useful for investigation of AAA pathogenesis have been developed. These models include infusion of Ang II into either *apolipoprotein E* (*ApoE*)$^{-/-}$ or *low-density lipoprotein receptor* (*Ldlr*)$^{-/-}$ mice. The Ang II/BAPN co-infusion model used in the present study is a novel models with morphological and histological characteristics similar to human AAA [22,27]. Ang II induces hypertension, oxidative stress, and inflammation [28], and BAPN, a lysyl oxidase inhibitor, induces degeneration of elastic laminas. Consequently, co-treatment with Ang II and BAPN has been speculated to cause a higher incidence of AAA formation. Another feature of this model is the use of C57BL/6-background WT mice, which eases the interpretation of the results as compared with using genetically engineered mice, such as *ApoE*$^{-/-}$ or *Ldlr*$^{-/-}$ mice.

Similar to IL-1β, IL-18 is synthesized as a biologically inactive precursor molecule. Assembly of the NLRP3 inflammasome leads to caspase-1-dependent maturation and release of the proinflammatory cytokines IL-1β and IL-18 [29]. Additionally, overexpression of IL-18 by intravenous injection of an adenoviral vector reportedly induces vascular inflammation and remodeling in a rat model of metabolic syndrome [30], decreases intimal collagen content and promotes vulnerable plaque phenotype in *ApoE*$^{-/-}$ mice [31]. A previous study reported that endogenous inhibition of IL-18 by IL-18-binding protein prevents fatty streak development and macrophage infiltration in the aorta of *ApoE*$^{-/-}$ mice [21]. By contrast, administration of exogenous IL-18 promotes atherogenesis accompanied by enhanced lipid and macrophage accumulation in the aortic lesion [32]. However, little is known about the role of IL-18 in the pathological process of AAA formation. To the best of our knowledge, this study is the first to demonstrate that IL-18 deletion attenuated AAA formation by inhibiting macrophage infiltration, inducing a switch in macrophage phenotype from proinflammatory M1 state to anti-inflammatory M2 state, and activating MMPs in the abdominal aorta.

Ang II reportedly induces IL-18 levels and enhances IL-18-mediated inflammatory gene expression in VSMCs [33–35]. Consistent with these findings, we observed that expression of *Il-18* and infiltration of F4/80-positive macrophages in the abdominal aorta were enhanced in Ang II/BAPN-induced AAA model mice as compared with sham mice. IL-18 is involved in inflammatory immune responses through the induction of chemokines, such as monocyte chemoattractant protein-1, in macrophages [36]. Additionally, IL-18 induces OPN expression in cardiac fibroblasts [17]. CD44 and integrins are cell surface adhesion receptors that play an important role in regulating cell migration by mediating interactions between the ECM and the actin cytoskeleton [37,38]. Moreover, OPN induces macrophage migration through interaction with CD44, α_4 and α_9 integrins [39], and OPN gene disruption results in reduced incidence of Ang II-induced AAA formation associated with decreased MMP-2 and MMP-9 activity [18]. In agreement with these observations, we showed that *Opn* expression and MMP activity in the abdominal aorta were attenuated in *Il-18*$^{-/-}$ mice compared with WT mice. OPN is usually expressed in preosteoblasts, osteoblasts, osteocytes, VSMCs, and endothelial cells. During the formation of atherosclerotic plaques, OPN is highly expressed in macrophages, VSMCs, and endothelial cells [14]. In the present study, Opn expression in the abdominal aorta of Ang II/BAPN-induced AAA-model mice was colocalized with VSMCs, suggesting that VSMCs are the main source of OPN. Moreover, our *in vitro* study revealed that IL-18 stimulation induces the enhanced *Opn* mRNA levels in VSMCs.

Several studies indicate that IL-18 is involved in atherosclerosis through various putative mechanisms [21,40,41]. IL-18 acts with IL-12

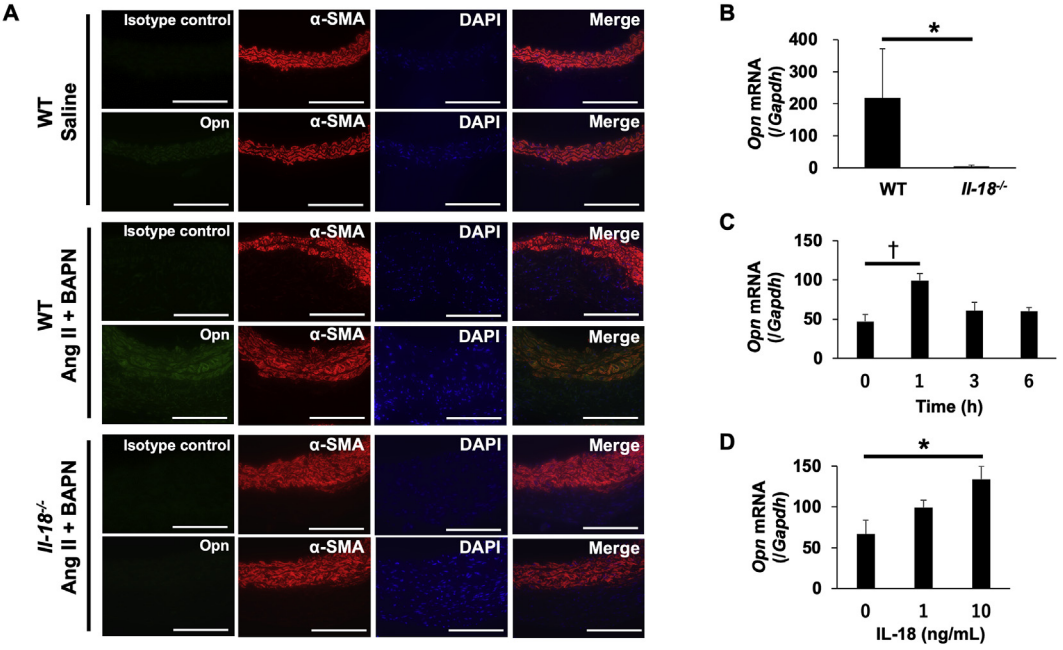

Fig. 4. Interleukin (IL)-18 deletion attenuates the osteopontin (OPN) expression in abdominal aortic aneurysm.
(A) Representative images of immunohistochemical staining for Opn, α-smooth muscle actin (α-SMA), and 4′-6-diamidino-2-phenylindole (DAPI) (nuclear stain) in wild-type (WT) and *Il-18*$^{-/-}$ mice abdominal aortas after saline or angiotensin II (Ang II) plus β-aminopropionitrile (BAPN) infusion. Scale bar, 100 μm. (B) Bar graph showing *Opn* expression in abdominal aortas from WT and *Il-18*$^{-/-}$ mice after Ang II plus BAPN infusion (n = 4 per group). All values are presented as the mean ± standard error of the mean. *$p < 0.05$. Data were analyzed using Mann-Whitney *U* test. (C) Bar graph showing the time course of *Opn* mRNA levels in rat aortic smooth muscle cells after treatment with 1 ng/mL IL-18 (n = 5 per group). All values are presented as the mean ± standard error of the mean. †$p < 0.01$. Data were analyzed using analysis of variance, followed by the Tukey post-hoc test. (D) Bar graph showing the dose-dependence of IL-18-induced *Opn* mRNA levels in rat aortic smooth muscle cells (n = 5 per group). Rat aortic smooth muscle cells were stimulated with the indicated concentrations of IL-18 for 1 h. All values are presented as the mean ± standard error of the mean. *$p < 0.05$. Data were analyzed using analysis of variance, followed by the Tukey post-hoc test.
Gapdh, glyceraldehyde 3-phosphate dehydrogenase.

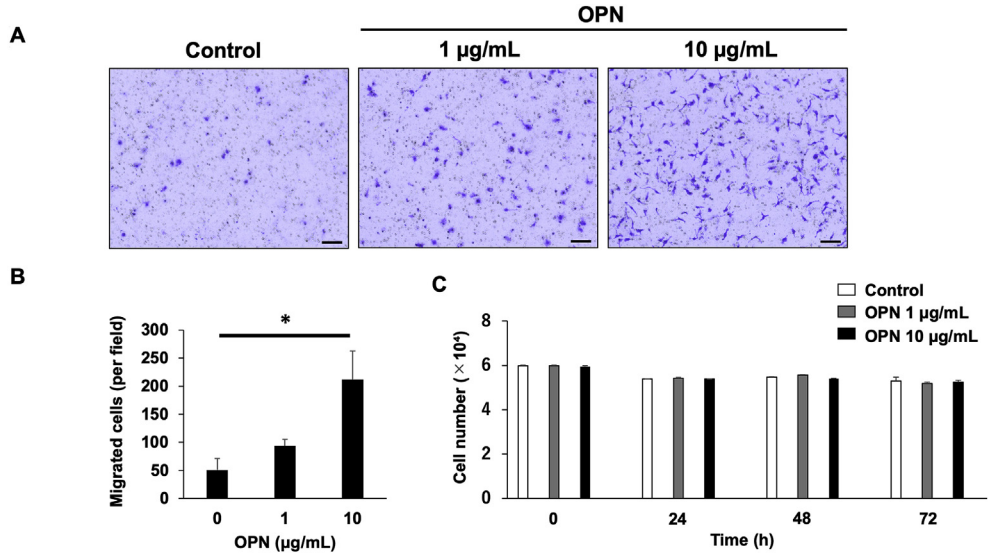

Fig. 5. Osteopontin (OPN) induces macrophage migration but not proliferation.
(A) Representative images of migrated wild-type (WT) peritoneal macrophages 24 h after OPN stimulation. Scale bar, 100 μm. (B) Quantification of migrated macrophages (n = 3 per group). (C) WT peritoneal macrophages were plated in 96-well plates at an initial density of 5×10^4 cells/well and cultured with 0.1% serum containing medium with or without OPN for 0, 24, 48, and 72 h. Cell proliferation was measured using a Cell Counting Kit-8 assay (n = 3 per group). All values are presented as the mean ± standard error of the mean. *$p < 0.05$. Data were analyzed using analysis of variance, followed by the Tukey post-hoc test.

to synergistically induce the production of interferon (IFN)-γ in T cells, natural killer cells, and macrophages. IFN-γ promotes atherosclerosis by activating macrophages, stimulating inflammatory cytokines, activating MMPs, and inhibiting collagen synthesis [33,42]. However, some conflicting opinions exist regarding the role of IFN-γ in AAA formation. Zhou et al. [43] and Xiong et al. [44] reported that IFN-γ directs the inflammatory cascade and promotes AAA in an elastase-induced AAA model. These observations support our hypothesis that IL-18 (IFN-γ-inducing factor) contributes to AAA pathogenesis. However, Shimizu et al. [45] and King et al. [46] demonstrated that IFN-γ protects against aneurysm formation and rupture. Because IL-18 plays an IFN-γ-independent proinflammatory role, this likely explains the different phenotypes observed between *Il-18*$^{-/-}$ mice and *Ifn-γ*$^{-/-}$ mice. These apparently conflicting results might be attributed to the difference in the AAA mouse models (whole-body IFN-γ-deficient mice vs. IFN-γ deficiency in *Cd43*$^{-/-}$ mice vs. *Il-18*$^{-/-}$ mice). Moreover, IFN-γ might exhibit a stimulatory or inhibitory effect on AAA formation, depending on the expression levels of the molecule.

There are several limitations to this study. First, our murine model is an acute model of AAA formation that differs from human chronic

aortic aneurysm. The precise role of IL-18 in all phases of vascular remodeling during aneurysm formation should be explored in further studies. Second, we did not focus on the incidence and pathophysiological mechanisms of thoracic aortic aneurysms. Aortic aneurysms are classified according to their anatomical location: thoracic or abdominal. Classically, a vast majority of AAAs have been considered atherosclerotic in origin and associated with inflammatory cell infiltration. By contrast, thoracic aortic aneurysms have been associated with mutations in genes encoding components of the VSMC contractile apparatus, the ECM, and key molecules associated with transforming growth factor-β signaling [11]. Thoracic aortic aneurysms frequently result from a non-inflammatory process of cystic medial degeneration, including VSMC loss and elastin fragmentation [47]. Although thoracic and abdominal aortic aneurysms share some common pathological phenotypes, including destruction of the ECM and loss of VCMCs, they are two distinct diseases. Therefore, the role of IL-18 in thoracic aortic aneurysms remains to be determined in future studies. Finally, mouse peritoneal macrophages may not represent the macrophage population in the aortic aneurysmal wall. Although no direct evidence has been provided to date, it has been suggested that most macrophages in the aortic aneurysmal wall derive from circulating monocytes [7]. Therefore, the precise role of OPN in circulating monocyte immigration into the aortic wall and local proliferation of the recruited monocytes remains unknown.

AAA is a common disease, and rupture of an aortic aneurysm results in severe mortality and morbidity. Currently, detailed mechanisms of AAA formation remain elusive, and the optimal medical therapy that controls AAA expansion has not yet been developed. In this study, we demonstrated that IL-18 plays an important role in the pathogenesis of AAA formation. Co-infusion of Ang II/BAPN enhanced Il-18 expression and subsequent Opn expression in VSMCs in our AAA mouse model. Additionally, our results suggested that the OPN contributes to macrophage infiltration and MMP activation in the abdominal aorta. Moreover, this IL-18/OPN-mediated pathway also altered macrophage polarization from an anti-inflammatory M2-to a proinflammatory M1-dominant phenotype. Furthermore, genetic deletion of *Il-18* resulted in reduced incidence of AAA formation, which was associated with attenuation of OPN-induced macrophage infiltration and MMP activation. Our findings provide important and novel insights into the pathogenesis of AAA formation and suggest that targeting IL-18-mediated pathways might hold therapeutic potential to prevent AAA.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

Tomoki Sakaue conceived the study and designed the experiments, performed the experiments and analyzed the data. Jun Suzuki and Jun Aono supervised the experiments and edited the manuscript. Osamu Yamaguchi, Takafumi Okura, Shuntaro Ikeda, Teruyoshi Uetani, and Tomoaki Nagao supervised the experiments. Mika Hamaguchi and Kayo Takahashi performed experiments. Haruki Okamura kindly provided *IL-18*$^{-/-}$ mice and supervised the experiments. Chika Suehiro designed and performed the experiments, analyzed the data, and drafted the manuscript.

Acknowledgments

We are grateful to Mr. Akihiro Umakoshi and Dr. Junya Tanaka from the Department of Molecular and Cellular Physiology, Ehime University Graduate School of Medicine, for their suggestions and help with the cell migration assay. We are also grateful to the staff of the Integrated Center for Science of Ehime University for assistance with animal care.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.08.003.

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