PPARγ agonist rosiglitazone alters the temporal and spatial distribution of inflammation during abdominal aortic aneurysm formation

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Abstract. Research into inflammation during abdominal aortic aneurysm (AAA) formation remains inconclusive. The present study aimed to demonstrate the temporal and spatial distribution of inflammatory cytokines, and to confirm the effect of peroxisome proliferator-activated receptor γ (PPARγ) on the incidence of AAA formation and the distribution of inflammation in the disease process. Male apolipoprotein $E^{-/-}$ mice were randomly divided into eight groups: Angiotensin II (Ang-II)-only 7, 14, 21, 28 and 42 days groups, Ang-II with rosiglitazone (RGZ) 28 and 42 days groups, and the saline control 42 days group. The early stage was defined as between 7 and 21 days, and the late stage as between 28 and 42 days. Incidences of early rupture and late rupture, aneurysm formation and the maximum diameters of the aorta were recorded. Suprarenal abdominal aortic tissues were collected for histological analysis, and western blotting was performed to reveal the distribution of inflammation. Treatment with Ang-II caused a significant dilation of the aorta in the late stage; however, this was not observed in the early stage. RGZ reduced the maximum diameters in the late stage. With the pathological process alterations, the inflammatory type shifted. Regarding temporal distribution, the tumor necrosis factor (TNF)-α expression level was increased over time, and the interleukin (IL)-10 expression level significantly decreased. When considering the spatial distribution, TNF-α was expressed dominantly in the aneurysmal body and IL-10 was dominant in the aneurysmal neck in the late stage. The PPARγ agonist RGZ may reduce the expression of TNF-α in the late stage and increase the expression level of IL-10, maintaining the TNF-α or IL-10 expression levels at the same levels as in the early stage. Aortic inflammation during AAA formation is dynamic. Protective anti-inflammatory cytokines are upregulated in the early 'compensatory stage'; however, pro-inflammatory cytokines are dominant in the late 'decompensatory stage'. PPARγ is likely to continue to upregulate the expression of anti-inflammatory cytokines, extend the 'compensatory stage', and decelerate the process of AAA development and rupture.

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Introduction

With the aging population, the number of patients with abdominal aortic aneurysm (AAA) is increasing yearly. The incidence of AAA may be 2-8.9% in the general population, and as high as 19.8% among men over the age of 75 (1). The pathological and physiological processes of AAA have been a continuous focus of research. The typical and common pathological alterations include: i) Infiltration of inflammatory cells (including macrophages, neutrophil granulocytes, T cells, B cells, mastocytes and natural killer cells) in the tunica intima, media and externa; ii) angiogenesis in the tunica media and apoptosis of vascular smooth muscle cells (VSMCs); and iii) degradation of the extracellular matrix, breakage of elastic fibers and loss of collagen. All of these factors indicate that inflammation may be the common mechanism in AAA formation, although the degree and type may vary in different types of AAA. Inflammation exists throughout the entire AAA process (2).

In particular, multiple inflammatory cytokines have been identified in aortic aneurysms. There is evidence that Th1- and Th2-type cytokines are involved in aneurysm formation (2). Schönbeck *et al* (3) identified that Th2-characteristic cytokines, including interleukin (IL)-4, IL-5 and IL-10, were predominantly expressed in the AAA tissue; however, IL-2 and IL-15, as Th1-characteristic cytokines, were expressed at low levels. However, other studies have demonstrated that Th1-associated cytokines are required for aneurysm formation. For example, it was observed that tumor necrosis factor (TNF)-α, IL-1β, IL-8 and C-C motif chemokine 2 have upregulated expression in AAA tissues (2). In animal models, interferon-γ (IFN-γ) is required in the process of aneurysm development involving

increased matrix metalloproteinase (MMP) expression (4). These differences may be attributed to differences in the source of the control tissue, patient demographics, lesion characteristics, the stage of aneurysm formation, and tissue preservation or treatment conditions (5). There is a hypothesis that persistent inflammatory conditions initiate the promotion of Th1-type inflammation, leading to atherosclerosis and fibrotic lesions; and the secondary trigger, including smoking, reactive oxygen species, autoimmune factors or genetic predisposition, may lead to conversion to a Th2-type cytokine profile, causing matrix degradation and ultimately, aneurysm formation (6).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily. In total, three types of PPARs have been discovered: PPAR-α, β and γ. They serve an important role in regulating energy metabolism and the circadian rhythm (7). PPARγ activators, thiazolidinediones (TZDs), are used in pharmaceutical therapy for diabetes. However, PPARγ has additionally been observed to be involved in the shift in cytokine production and inflammation. TZDs may reduce TNF-α expression in sepsis, ischemia/reperfusion, gastric injury and spinal trauma models (8). PPARγ agonists may reduce the expression of TNF-α and MMP-9 in the aortic aneurysmal wall and retroperitoneal periaortic fat (9). The expression of other Th1-associated cytokines, including IL-6 and IL-1β, is additionally downregulated by PPARγ agonists in sepsis or ischemia/reperfusion models (8). However, PPARγ affects the expression of Th2-type inflammatory cytokines. Rosiglitazone (RGZ), a PPARγ agonist, induces IL-10 expression in colitis and Parkinson's disease models (10,11).

PPARγ has been demonstrated in certain studies to be involved in AAA formation and development. TZDs reduced the maximum diameter and rupture of the aorta in an Ang-II-induced experimental aneurysm model of apolipoprotein E (ApoE)$^{-/-}$ mice (7,12). However, in aneurysms of other parts of the body, including cerebral aneurysm, pioglitazone was not able to reduce the incidence of aneurysm (13).

The distribution of Th1 and Th2-type inflammation in the process of aortic aneurysm formation and development has not yet been confirmed, and one of the aims of the present study was to demonstrate the spatial and temporal distributions of different types of inflammatory cytokines. Therefore, TNF-α and IL-10 were selected for examination, as they are typical examples of Th1- and Th2-type cytokines. Additionally, the present study attempted to confirm the effect of PPARγ on the incidence of AAA formation and on the distribution of inflammation in the disease process.

Materials and methods

Animals and treatment. Male ApoE$^{-/-}$ mice (8-10 weeks old; weight 17-23 g; n=80) were obtained from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), housed in clean barrier conditions (22-25˚C, 1 bar pressure, 12-h light/dark cycle, and free accessing to water and food), and were administered a standard laboratory diet in the Peking Union Medical College Hospital (Beijing, China) animal house. The experiments in the present study were approved by the Ethics Committee of Peking Union Medical College Hospital. All of the 80 mice were divided into eight groups (n=10 mice/group): i) Ang-II 7 days group; ii) Ang-II 14 days group; iii) Ang-II 21 days group; iv) Ang-II 28 days group; v) Ang-II 42 days group; vi) Ang-II+RGZ 28 days group; vii) Ang-II+RGZ 42 days group; and viii) saline control 42 days group. Osmotic pumps (ALZET Osmotic Pumps, Cupertino, CA, USA) delivering 1,000 ng/kg/min Ang-II (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 7, 14, 21, 28 or 42 days were implanted subcutaneously in the seven groups of mice. Ang-II was replaced by saline in osmotic pumps in the saline control group. In groups 6 and 7, intragastric administration of RGZ (3 mg/kg/day) was begun 7 days prior to osmotic pump implantation and ended when the mice were sacrificed. When it was time for tissue harvesting, the mice were sacrificed. The tissues of the suprarenal aortas were removed and prepared for further analysis. According to a previous study (7), AAA was able to develop in 28 days in this animal model. For analysis, 7-21 days was defined as the early stage, and 28-42 days as the late stage. For AAA rupture, a rupture that occurred in the first 7 days following Ang-II pumping was defined as an early rupture and the remainder as late ruptures.

Histology. The maximum diameters of the suprarenal abdominal aortas were measured, and the widest parts of suprarenal abdominal aortas were fixed in 10% formalin for 24 h at room temperature. If aneurysm formation (compared with the normal regions, dilation ≥50%) was observed, the aneurysm neck (defined as 0.5 cm of the normal aorta above the aneurysm) was additionally reserved. The fixed samples were embedded in paraffin, sectioned into 10-μm-thick slices and then stained with hematoxylin and eosin to confirm the formation of AAA. The hematoxylin and eosin staining was performed according to standard protocols (14), and the stained slides were observed using a DMI4000 B light microscope (Leica Microsystems, Inc., Buffalo Grove, IL, USA). Images were captured 50 times (magnification, x50).

Western blot analysis. A portion of the widest part of the suprarenal abdominal aorta of each mouse was stored at -80˚C, in addition to the aneurysm neck if there was aneurysm formation. The aortic proteins were subsequently extracted with protein lysis buffer (Ukzybiotech., Ltd., Beijing, China; cat. no. P0001). The protein concentration was standardized with a Bio-Rad protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Equal amounts (15 μg) of aortic extracts from mice in the different groups were loaded onto a 15% SDS-PAGE gel and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% skim milk in Tris-buffered saline with Tween 20 (0.01 mol/l Tris-HCl, pH 7.6, 0.15 mol/l NaCl, 0.05% Tween 20) for 1 h at room temperature. The membranes were incubated with a mouse anti-TNF-α monoclonal antibody (Ab; ProteinTech Group, Inc., Chicago, IL, USA; cat. no. 60291; 1:500) or rabbit anti-IL-10 polyclonal Ab (Lifespan Biosciences, Inc., Seattle, WA, USA; cat. no. LS-C331959; 1:500) overnight at 4˚C. GAPDH was selected as the internal control (Cell Signaling Technology, Inc., Danvers, MA, USA; cat. no. 5174; 1:1,000). The bound primary Ab was detected with horseradish peroxidase-linked goat anti-mouse immunoglobulin

Table I. Aortic diameters and ruptures in mice.

Group	I	II	III	IV	V	VI	VII	VIII
Maximum diameter, cm	0.12±0.01	0.12±0.01	0.14±0.02	0.17±0.05	0.18±0.05	0.13±0.02	0.15±0.03	0.12±0.04
Aneurysm formation, no. cases	0	0	1	3	5	1	2	0
Early rupture, no. cases	0	0	1	1	1	1	2	0
Late rupture, no. cases	0	0	0	0	3	0	0	0

Maximum diameters are expressed as the mean ± standard error. I, Ang-IIx7 days; II, Ang-IIx14 days; III, Ang-IIx21 days; IV, Ang-IIx28 days; V, Ang-IIx42 days; VI, Ang-II+RGZx28 days; VII, Ang-II+RGZx42 days; VIII, saline control. Ang-II, angiotensin II; RGZ, rosiglitazone.

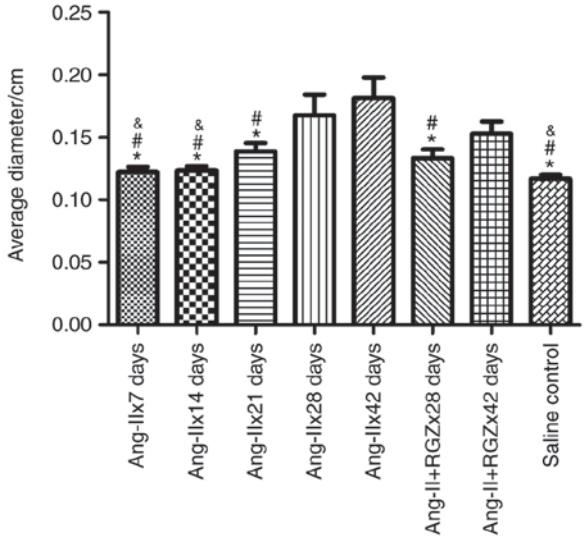

Figure 1. Average maximum diameters (cm) of abdominal aortas in all the groups. The diameters exhibited an increasing trend over time, and RGZ may inhibit aortic dilation. *P<0.05 vs. Ang-IIx28 days group, **P<0.01, and ***P<0.001; #P<0.05 vs. Ang-IIx42 days group, ##P<0.01, and ###P<0.001; &P<0.05 vs. Ang-II+RGZx42 days group. Ang-II, angiotensin II; RGZ, rosiglitazone.

G (IgG; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA; cat. no. 115-035-003; 1:10,000) or anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc.; cat. no. 111-035-003; 1:10,000), respectively. The secondary incubation was conducted at room temperature for 40 min. The enhanced chemiluminescent method (EMD Millipore, Billerica, MA, USA; cat. no. WBKLS0500) was subsequently used to develop the bands. The gray value of TNF-α or IL-10 was divided by the gray value of the internal reference to calculate the relative gray value. The gray value was calculated using Image J 1.50i software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. All of the data were analyzed using SPSS version 19.0.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). The results are presented as the mean ± standard error. Differences were analyzed using Student's unpaired t-test or analysis of variance followed by the Newman-Keuls multiple comparisons test. P<0.05 was considered to indicate a statistically significant difference. All experiments were repeated at least three times.

Table II. Contrast between the late stages without or with RGZ.

Group	Late stage without RGZ (%)	Late stage with RGZ (%)	P-value
Aneurysm formation	47.1	18.8	0.141
Early rupture	10.5	15.8	0.631
Late rupture	17.6	0.0	0.227

RGZ, rosiglitazone.

Results

RGZ reduces the diameters of the aortas in Ang-II-treated ApoE$^{-/-}$ mice. A mouse succumbed to pneumorrhagia during intragastric gavage in the Ang-II+RGZx42 days group, and one mouse in each of the Ang-IIx14 days, Ang-IIx42 days and saline control groups succumbed to anesthetization. There was one case of the pump falling out in the Ang-IIx7 days group. Compared with the saline control group, the maximum diameters of the Ang-IIx7 days, Ang-IIx14 days and Ang-IIx21 days groups were not significantly different (P=0.676, 0.616 and 0.098, respectively). Treatment with Ang-II resulted in dilation of the aortas in ApoE$^{-/-}$ mice, primarily in the late-stage groups; the Ang-IIx28 days (P<0.001) and Ang-IIx42 days groups (P<0.001). RGZ reduced the maximum diameters of the aortas. In the 28 days group, RGZ significantly prevented dilation of the aortas (Ang-IIx28 days, 0.17±0.05 cm vs. Ang-II+RGZx28 days, 0.13±0.02 cm; P=0.012); in the 42 days group, use of RGZ had a trend of reducing the maximum diameters (Ang-IIx42 days, 0.18±0.05 cm vs. Ang-II+RGZx42 days, 0.15±0.03; P=0.055). When comparing the entire late stage, RGZ significantly reduced the maximum diameters of the aortas in the Ang-II-induced AAA model of ApoE$^{-/-}$ mice (without RGZ, 0.17±0.05 cm vs. with RGZ, 0.14±0.02 cm; P=0.021; Fig. 1 and Table I).

However, there was no aneurysm formation in the control group or in the early stage, with the exception of one case that was identified in the Ang-IIx21 days group. In the late stage, aneurysm formation was significant compared with the control and early stage (late stage 47.1% vs. control 0%; P=0.012; and vs. early stage 3.7%; P=0.002; Table I). Early rupture was not influenced by RGZ (Ang-II, 10.5% vs. Ang-II+RGZ, 15.8%; P=0.631; Table II). There was also no statistically significant difference for aneurysm formation or rupture in the late stage

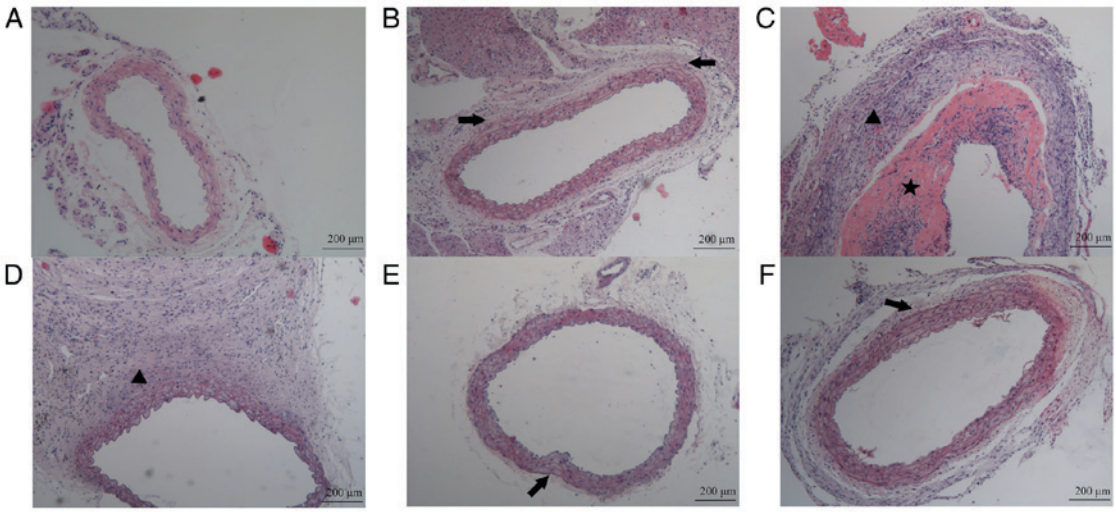

Figure 2. Different pathological features in Ang-II-treated apoplipoprotein $E^{-/-}$ mice with or without RGZ administration (magnification, x50). (A) Saline-infused control; (B) early stage, Ang-II-infused for 14 days; (C) late stage (aneurysmal body), Ang-II-infused for 28 days; (D) late stage (aneurysmal body), Ang-II-infused for 42 days; (E) late stage (aneurysmal neck), Ang-II-infused for 28 days; (F) late stage with RGZ, Ang-II-infused for 28 days with RGZ administration. Ang-II, angiotensin II; RGZ, rosiglitazone. The arrows indicate early alterations of elastic fibers. The triangle indicates thickening and inflammatory infiltration of aortic wall and the star indicates blood clots in the lumen of aorta.

of aneurysm development between the groups with RGZ and without RGZ. The rates of aneurysm formation in the late stage were 47.1 and 18.8% in Ang-II without RGZ groups and Ang-II with RGZ groups, respectively (P=0.141). Except for two mice that succumbed and one mouse that had claudication in the Ang-IIx42 days group, no mice succumbed or presented with lower extremity ischemia due to the late rupture of aneurysms in the other groups. In the late stage, the incidences of rupture were 17.6% and 0 in the groups without RGZ and with RGZ, respectively (P=0.227).

Pathomorphology of the aorta in Ang-II-treated ApoE$^{-/-}$ mice. The saline-infused control mice had a normal aortic structure without aortic wall thickening, fiber breakage or inflammatory cell infiltration (Fig. 2A). However, early alterations in Ang-II-treated mice primarily focused on the fiber arrangement. The elastic fibers became partially straight and the space between the fibers increased (Fig. 2B). By the late stage of aneurysm formation, the aortic structure at the aneurysmal body was notably damaged, and the boundaries of the intima, media and adventitia became indistinct. The aortic wall was thickened; inflammatory cells infiltrated markedly, particularly in the adventitia; and the VSMCs became disordered or disappeared. The fibers, or even the whole walls, were broken down. Blood clots formed in the lumen of the aorta (Fig. 2C and D). However, the structure at the aneurysmal neck was quite similar to that in the early stage of Ang-II infusion (Fig. 2E). RGZ may inhibit the occurrence of the late pathological alterations. Although the wave shape of the elastic fibers may have altered to a linear type, the aortic structure remained intact and the inflammatory cell infiltration was unremarkable (Fig. 2F).

Temporal distribution of inflammatory types during AAA development. In the present study, it was observed that the expression of Th1- and Th2-associated inflammatory factors altered during the development of aortic aneurysms in Ang-II-induced ApoE$^{-/-}$ mice. Th1-associated cytokine TNF-α expression increased in the aortic tissue over time; whereas, Th2-associated cytokine IL-10 expression decreased (Fig. 3A). Therefore, Th2-type inflammation served a dominant role in the early stage of aneurysm formation, and Th1-type in the late stage.

The average relative gray values of TNF-α (Fig. 3B) in the Ang-IIx21 days group, Ang-IIx28 days group and Ang-IIx42 days group were significantly increased compared with the Ang-IIx7 days group (P=0.018, 0.004 and <0.001, respectively); however, compared with the Ang-IIx14 days group, the average relative gray values of TNF-α were not significantly different, apart from in the Ang-IIx42 days group (P=0.602, 0.260 and 0.047, respectively). There was no significant difference between the Ang-IIx7 days group and Ang-IIx14 days group (P=0.053), or between the Ang-IIx28 days group and Ang-IIx42 days group (P=0.349). The average relative gray values of IL-10 (Fig. 3C) in the late-stage groups were significantly decreased compared with the early-stage groups (28 vs. 7 days, P=0.004; 28 vs. 14 days P=0.004; 42 vs. 7 days, P=0.010; 42 vs. 14 days, P=0.010). However, there were no significant differences between the average relative gray values of IL-10 of the Ang-IIx7 days group and Ang-IIx14 days group (P=0.996) or between the Ang-IIx28 days group and the Ang-IIx42 days group (P=0.690). There was no significant difference between Ang-IIx21 days and other groups.

Spatial distribution of inflammatory types. In mice with aneurysm formation in the late stage, the differential expression of TNF-α and IL-10 in the aneurysmal body tissue and aneurysmal neck tissue was compared. As demonstrated in Fig. 3D, the expression of TNF-α was increased in the aneurysmal body compared with the saline control, and decreased in the aneurysm neck compared with the saline control. The opposite results were observed for the expression of IL-10.

The expression of TNF-α was increased at the site of the aneurysmal body compared with the control (P=0.016):

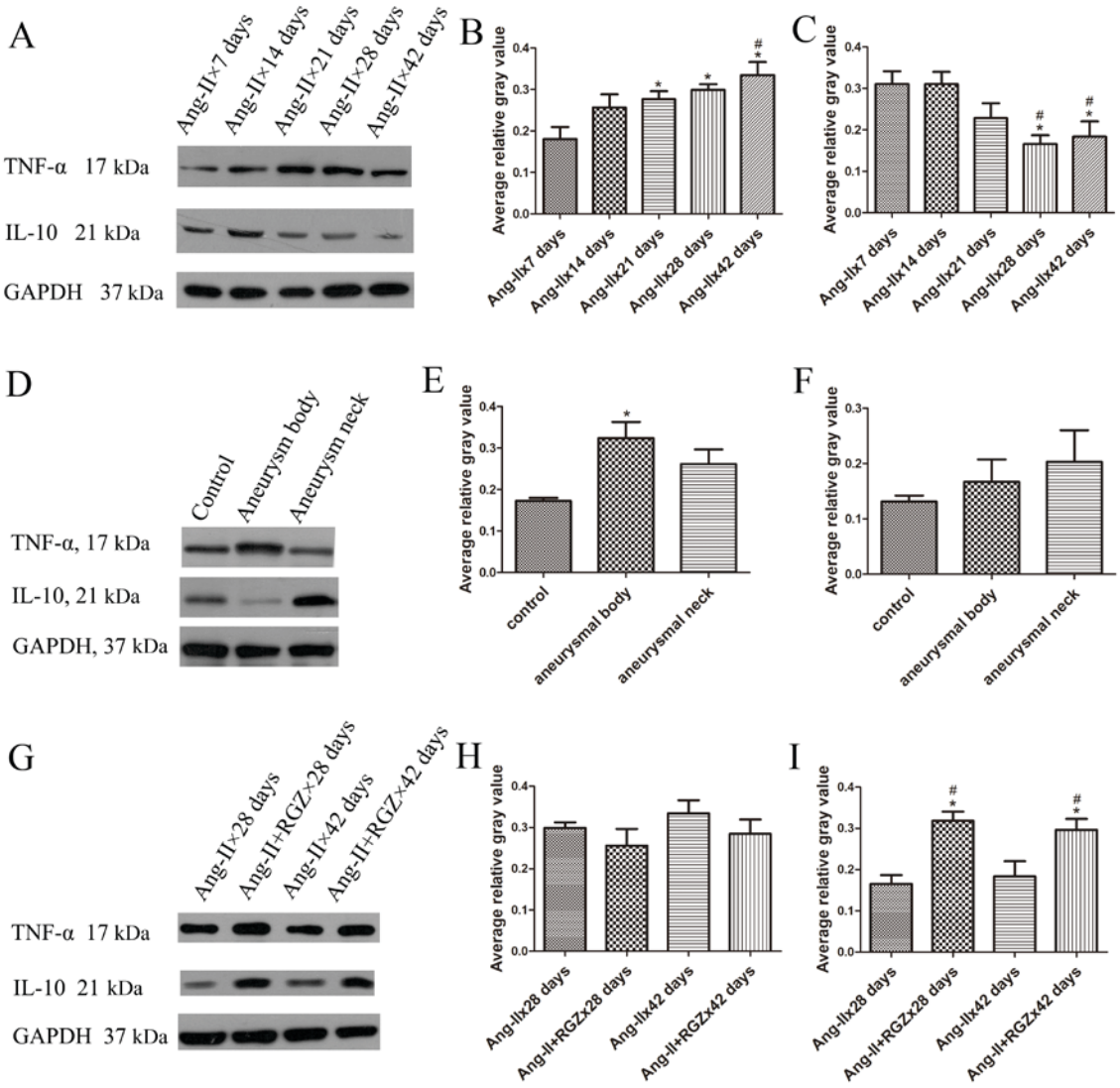

Figure 3. Alteration in the expression of Th1- and Th2-associated inflammatory factors during the development of AAA. (A) Expression of TNF-α and IL-10 during AAA development. (B) Average relative gray values of TNF-α at different times. (C) Average relative gray values of IL-10 at different times. *P<0.05, **P<0.01 and ***P<0.001 vs. Ang-IIx7 days group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. Ang-IIx14 days group. (D) Expression of TNF-α and IL-10 in the aneurysmal body and neck in the late stage of AAA formation. (E) Average relative gray values of TNF-α at different sites. (F) Average relative gray values of IL-10 at different sites. *P<0.05 vs. control. (G) RGZ reduced TNF-α expression and upregulated IL-10 expression in the late stage of AAA formation. (H) Alteration in the average relative gray values of TNF-α following administration of RGZ. (I) Alteration in the average relative gray values of IL-10 following administration of RGZ. *P<0.05 vs. Ang-IIx28 days group, and **P<0.01. $^{\#}$P<0.05 vs. Ang-IIx42 days group, and $^{\#\#}$P<0.01. AAA, abdominal aortic aneurysm; TNF, tumor necrosis factor; IL, interleukin; Ang-II, angiotensin II; RGZ, rosiglitazone.

However, there was no significant difference between the body and neck sites (P=0.211), or between the neck site and the control (P=0.109; Fig. 3E). The expression level of IL-10 at the site of the aneurysmal neck exhibited increased expression compared with the site of the aneurysmal body (P=0.573) or in the control (P=0.312). There was no marked difference between the expression level of IL-10 in the aneurysmal body and the control (P=0.607; Fig. 3F).

PPARγ agonist RGZ promotes Th2-associated inflammation in AAA. RGZ was able to reduce the TNF-α expression level and increase the expression level of IL-10 in the aortic tissue (Fig. 3G). During the development of AAA, the expression level of TNF-α increased over time as mentioned above; however, RGZ was able to reverse this trend of upregulation in the late stage of AAA development. The expression level of TNF-α in the Ang-II+RGZ groups was decreased compared with the Ang-II groups (Ang-IIx28 days vs. Ang-II+RGZx28 days, P=0.350; and Ang-IIx42 days vs. Ang-II+RGZx42 days, P=0.282; Fig. 3H). The effect of RGZ on the expression of IL-10 was more marked (Fig. 3I). Compared with the Ang-II groups, the IL-10 expression level in the Ang-II+RGZ groups was increased (Ang-IIx28 days vs. Ang-II+RGZx28 days, P=0.001; and Ang-IIx42 days vs. Ang-II+RGZx42 days, P=0.010).

The results above demonstrated that: i) Th2-type and Th1-type inflammatory cytokines are involved in AAA formation and they dominate, in turn, during AAA development in Ang-II-induced ApoE$^{-/-}$ mice; ii) the inflammatory types in the aneurysmal body and neck are different; iii) the PPARγ agonist, RGZ, may reduce the dilation of aortas induced by Ang-II in ApoE$^{-/-}$ mice and may prevent AAA late rupture to a

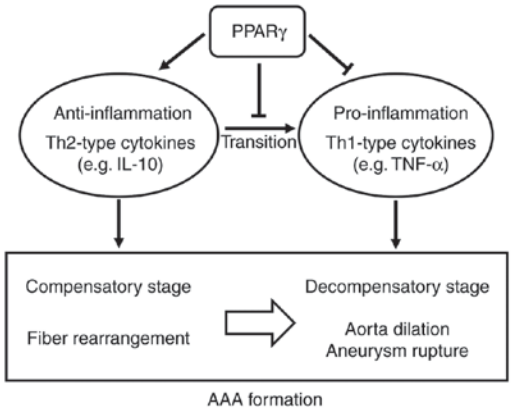

Figure 4. Hypothetical schematic of inflammation transition in AAA formation, and the mechanism through which PPARγ may inhibit AAA. AAA, abdominal aortic aneurysm; PPARγ, peroxisome proliferator-activated receptor γ; IL, interleukin; TNF, tumor necrosis factor.

certain degree; and iv) the shift in inflammatory types may be one of the mechanisms that allows RGZ to prevent the formation of AAA (Fig. 4).

Discussion

There has been evidence indicating that the renin Ang system serves an important role in human AAA (15,16). More than a decade ago, it was confirmed that Ang-II was able to promote atherosclerotic lesions and abdominal aortic aneurysm formation in ApoE$^{-/-}$ mice (17). The pathological alterations in abdominal aortas in Ang-II-infused ApoE$^{-/-}$ mice were consistent with a previous report (18). In the past, it was thought that Ang-II promoted pathological processes in the aorta via two primary mechanisms: By increasing arterial blood pressure, and by stimulating monocyte recruitment and activating macrophages (19-21). However, certain previous studies reported that the effect of producing AAA by Ang-II is independent of elevations in blood pressure, or alterations in plasma lipid and cholesterol concentrations (17,22,23). However, increasing evidence has demonstrated that an Ang-II infusion may promote leucocyte infiltration, inflammatory responses, extracellular matrix degradation and vascular oxidative stress (24), and that these effects of Ang-II may occur primarily through the type I Ang-II receptor (25).

As key mediators of inflammation, cytokines serve an important role in a number of chronic diseases; however, they have not been sufficiently studied in the context of AAA. Traditionally, T cell-derived cytokines are classified into two types, the Th1 type and Th2 type. Th1-type cytokines may activate macrophages and T cells and produce strong immune responses, while Th2-type cytokines produce relatively milder immune responses (26). IFN-γ and TNF-α, termed pro-inflammatory factors, are typical examples of Th1-type cytokines, and their effects on AAA are unclear. Xiong *et al* (4) reported that IFN-γ was able to promote AAA formation in mice lacking CD4^{+} cells; however, King *et al* (27) identified that an IFN-γ deficiency was able to protect Ang-II-treated mice from AAA formation. It was additionally demonstrated that in the calcium chloride model, mice with a TNF-α deficiency were resistant to AAA formation (28); however, AAA formation was not significantly affected in Ang-II-treated low-density lipoprotein receptor$^{-/-}$ mice with a TNF-α receptor deficiency (29). Th2-type cytokines may additionally serve different roles in AAA. IL-4 and IL-5 were revealed to promote AAA (30,31), although they have the ability to prevent atherosclerosis (32,33). IL-10 is considered to be an anti-inflammatory cytokine and has the ability to suppress the activation of Th1 cells and macrophages (26). In IL10$^{-/-}$ mice, AAA was induced more easily by Ang-II (34). However, in an early study of human AAAs, Th2-type cytokines, including IL-10, IL-4 and IL-5, were expressed more predominantly in the AAA tissues compared with Th1-type cytokines (IL-2, IL-12, IL-15 and IL-18) (3).

Based on the above, there may be some confusion regarding these complex results. In reality, considering this question in a static way is not appropriate. As AAA development is a gradual, altering process, inflammation, including cells and cytokines, may alter. The two types of vascular diseases, atherosclerosis and aneurysm, are usually associated, and there is certain speculation regarding the mechanisms of transition from atherosclerosis to aneurysm, as previously mentioned (6). However, data from human or mouse studies has demonstrated that in atherosclerosis and AAA pathology, in the majority of cases, these conditions co-exist (26), and specific additional key factors, including genetics or smoking, may be required to trigger the formation of AAA (2,26). When considering AAA alone, the temporal and spatial distributions of inflammatory cytokines remain unclear. The present study demonstrated that in the Ang-II-induced AAA model of ApoE$^{-/-}$ mice, IL-10 was expressed predominantly in the early stage of AAA development; however, it was attenuated in the late stage. This is consistent with the results of a previous study (32). Conversely, the expression of TNF-α significantly increased between the early and late stages of AAA formation. Considering the alteration that was observed, it was hypothesized that anti-inflammatory Th2-type cytokines may be produced to resist the inflammation caused by the Ang-II infusion in the early stage and that this process may be regarded as the 'compensatory stage'. IL-10 may suppress TNF-α expression during this stage, and the infiltration of inflammatory cells may not be substantial. With inflammation-enhancing inflammatory cells, including macrophages, lymphocytes accumulate and pro-inflammatory Th1-type cytokines are increasingly expressed. In turn, TNF-α promotes inflammation in the local region, the production of MMPs, (35) and the destruction of aortic walls, leading to AAA formation. This action may be considered the 'decompensatory stage'. As the aneurysmal neck is the marginal area of AAA, there may be a 'delayed effect' in inflammation at the site of the aneurysmal neck. When the inflammatory type shifts to the 'decompensatory stage' in the aneurysmal body, the inflammatory type remains as that of the 'compensatory stage' in the aneurysmal neck. This is consistent with the previous opinion that different sampling sites may impact the inflammatory types of AAA (36).

The PPARγ agonist RGZ reduced aortic dilation and late rupture in the present study. This result is consistent with the results of a previous study conducted by Jones *et al* (7). However, in this early study, the administration of RGZ reduced the expression of TNF-α induced by Ang-II; however,

it exerted no marked effect on IL-10. In other models, PPARγ may induce the production of IL-10 (8,37,38). The difference between the present results and those of Jones *et al* (7) may be due to the experimental conditions, including the age of the mice (6-8 weeks in the present study compared with 12 months in the study conducted by Jones *et al* (7)). Different previous studies have demonstrated that PPARγ may influence inflammation, and the biggest focus has been the function of the shifting of monocyte subtypes. PPARγ may promote transformation to M2 macrophages, and TZDs are the most potent PPARγ ligands to induce M2 polarization (8,39). ILs, including IL-4, IL13 and IL-10, as Th2 types, may stimulate M2 macrophages, and M2 macrophages, in turn, may promote the production of anti-inflammatory cytokines and jointly create a microenvironment of tissue repair and wound healing (8). In the present study, the PPARγ agonist RGZ significantly upregulated IL-10 in the late stage and the expression level of IL-10 was comparable with the early stage. Considering the pathological alterations together, PPARγ may maintain the aortic inflammatory conditions in the 'compensatory stage' and delay the development and rupture of AAA. These results demonstrated that PPARγ serves a protective role in AAA formation induced by Ang-II in $ApoE^{-/-}$ mice.

In human AAA studies, research is limited to the late-stage of this disease. Although there are specific differences between the Ang-II-infused mouse model and humans, a number of primary pathological features of AAA in humans have been reproduced in this model (18). The present study preliminarily demonstrated that the overexpression of Th1- or Th2-associated cytokines is altered during the process of AAA formation and that they predominantly express, in turn, in the early compensatory stage anti-inflammatory factors, including IL-10, to suppress pro-inflammatory factors; whereas, in the decompensatory stage, pro-inflammatory cytokines, including TNF-α, are upregulated. PPARγ may aid the maintenance of the expression levels of anti-inflammatory cytokines and extend the compensatory stage (Fig. 4).

There are limitations to the present study. The small number of samples may influence the significance of the statistical differences, and only a few results demonstrated variation trends. Further research is required to inform a broader audience regarding pathology and inflammation during AAA formation, including inflammatory cell infiltration and the downstream factors of these cytokines. Another limitation of the present study is the lack of a direct mechanism through which PPARγ may act on the temporal and spatial distribution of inflammation. Additional research is required to focus on the direct mechanism, and PPARγ-knockout mice may be useful.

In conclusion, aortic inflammation during AAA formation is dynamic and variable when considering the temporal and spatial distributions. The overexpression and dominance of the relevant cytokines require examination at different stages. Protective anti-inflammatory cytokines are upregulated in the early compensatory stage; however, pro-inflammatory cytokines are dominant in the late decompensatory stage. When focusing on the inflammatory alterations in AAA, PPARγ is likely to continue to upregulate anti-inflammatory cytokines and decelerate the process of AAA development and rupture.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YC and WW conceived and designed the experiments. WW and RS performed the animal experiments. WW produced the manuscript. WW and RS conducted data analysis. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The experiments in the present study were approved by the Ethics Committee of Peking Union Medical College Hospital (Beijing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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