**Endogenous Interleukin-1β Is Implicated in Intraplaque Hemorrhage in Apolipoprotein E Gene Null Mice**

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**Background:** Intraplaque hemorrhage (IPH) has been implicated in plaque instability and rupture in atherosclerotic lesions, although the mechanisms by which IPH progresses remain largely unknown. In this study, apolipoprotein E-deficient mice with carotid artery ligation and cuff placement around the artery were used, and pro-inflammatory cytokines that are implicated in IPH were analyzed.

**Methods and Results:** The expression of interleukin-1β (IL-1β) increased significantly following cuff placement compared with mice with carotid artery ligation alone. IPH occurred in the cuff-placed carotid artery following treatment with the negative control (NC) small interfering RNA (siRNA). However, the occurrence was significantly reduced in the cuff-placed carotid artery following treatment with an IL-1β siRNA. Neovessel formation was significantly reduced in the carotid artery treated with the NC siRNA compared with that treated with IL-1β siRNA. IL-1β significantly inhibited the tube formation and wound healing capacities of vascular endothelial cells in vitro. Furthermore, immunostaining of matrix metalloproteinase-9 (MMP-9) significantly increased in the carotid artery treated with the NC siRNA compared with that treated with IL-1β siRNA.

**Conclusions:** These results suggest that endogenous IL-1β is implicated in the progression of IPH via the inhibition of physiological angiogenesis in the atherosclerotic plaque, leading to the formation of leaky neovessels. Furthermore, the stimulation of MMP-9 expression may also contribute to the formation of leaky neovessels.

**Key Words:** Angiogenesis; Interleukin-1β; Intraplaque hemorrhage; Matrix metalloproteinase-9

Plaque rupture of thin-cap fibroatheroma and erosion of plaques lacking a necrotic lipid core are major causes of coronary thrombosis that potentially results in acute coronary syndrome. Intensive studies have been performed to detect and inhibit the progression of vulnerable plaques. Among a variety of factors that are implicated in the progression of stable plaques to unstable plaques, intraplaque hemorrhage (IPH) is regarded as a critical factor that triggers the instability of plaques.

IPH occurs as a consequence of rupture and bleeding of immature plaque neovessels. The neovessels lack surrounding mural cells, and the endothelium of neovessels lacks intercellular gap junctions, with detached basement membranes. Extravasated erythrocytes from the leaky neovessels can be a source of cholesterol accumulation, as erythrocyte membranes are enriched in cholesterol. Heme and iron released from erythrocytes can generate reactive oxygen species that stimulate inflammation in the plaque lesions.

Apolipoprotein E gene (ApoE) knockout (KO) mice have been used as a model for plaque rupture with IPH. Johnson and Jackson detected plaque ruptures with IPH in the brachiocephalic artery of ApoE KO mice following a long period of high-fat diet feeding (46±3 weeks). Although spontaneous IPH and plaque rupture could be observed in this model, it took a long time to detect IPH and plaque rupture. Recently, Sasaki et al reported a model in which plaque rupture with IPH occurs in ApoE KO mice following carotid artery ligation and subsequent cuff placement. They detected a plaque rupture with IPH following ligation for 4 weeks and subsequent cuff placement for several days. We used this model and found IPH in more than 90% of ApoE KO mice 5 days after cuff placement.

Using this model, we found that interleukin-1β (IL-1β) expression remarkably increased among a variety of pro-inflammatory cytokines after cuff placement. In this study, we examined the functional significance of IL-1β in the triggering of IPH in this model.
IL-1β Is Implicated in Intraplaque Hemorrhages

after ligation. The carotid artery was harvested 5 days after cuff placement.

Real-Time Polymerase Chain Reaction (RT-PCR)
Total RNA was extracted from the carotid artery using NucleoSpin RNA II (Macherey-Nagel, Duren, Germany). TRizol Reagent (Life technologies, Tokyo, Japan) was used for the extraction of total RNA from cultured cells according to the manufacturer’s instructions. Total RNA was subjected to reverse transcription using a ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan). The expression of cytokines including angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), adrenomedullin (AM), vascular endothelial growth factor-A (VEGF-A), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) was examined by real-time polymerase chain reaction (PCR). The expression of these cytokines was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH; n=4 per group). *P<0.05 and **P<0.01 vs. control group (n=5 per group).

Methods

Reagents
The reagents used in this study are described in the Supplementary section.

Cell Culture
Human umbilical vein endothelial cells (HUVECs) were obtained from Sanko Junyaku Co., Ltd. (Tokyo, Japan), and cultured in HuMedia-EG2 (Kurabo, Osaka, Japan) that contained 2% fetal bovine serum (FBS), epidermal growth factor, basic fibroblast growth factor (bFGF), hydrocortisone, and heparin as growth factors. RAW264.7 cells and THP-1 cells were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) and were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% FBS and RPMI1640 medium containing 5% FBS respectively.

Animal Experiments
All animal studies were performed in accordance with the guidelines for animal care of the University of Tokyo. ApoE KO mice were obtained from the Jackson laboratory (Bar Harbor, ME, USA) and fed normal chow. The ligation and cuff placement of the carotid artery were performed, as previously described, with slight modifications.8 Mice were anesthetized with pentobarbital (50mg/kg body weight) by intraperitoneal injection. Following a midline cervical incision, the common carotid artery of 9-week-old ApoE KO mice was ligated with a nylon thread just proximal to its bifurcations. A polyethylene cuff (inner diameter: 580μm) was placed proximally to the ligation site 5 weeks after ligation. The carotid artery was harvested 5 days after cuff placement.

Figure 1. Ligation followed by cuff placement induces intraplaque hemorrhage (IPH) in the carotid artery. Representative hematoxylin and eosin (HE) staining of the carotid artery from apolipoprotein E (ApoE) knockout (KO) mice (Control), carotid artery with ligation alone (Ligation), and carotid artery with ligation plus cuff placement (Ligation_Cuff). Bars indicate 100μm.

Figure 2. Expression of cytokines in the carotid artery harvested from apolipoprotein E (ApoE) knockout (KO) mice. RNA was extracted from the carotid artery without any treatment (Cont), carotid artery with ligation alone (Lig), carotid artery with cuff placement alone (Cuff), and carotid artery with ligation plus cuff placement (Lig_Cuff). The expression of angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), adrenomedullin (AM), vascular endothelial growth factor-A (VEGF-A), interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) was examined by real-time polymerase chain reaction (PCR). The expression of these cytokines was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH; n=4 per group). *P<0.05 and **P<0.01 vs. control group (n=5 per group).
to examine the role of endogenous IL-1β expression in the formation of IPH, two synthetic small interfering RNAs (siRNAs: IL-1βsiRNA-1 and IL-1βsiRNA-2) that inhibit IL-1β expression and a synthetic siRNA that is predicted not to target any known vertebrate gene (negative control siRNA: NCsiRNA) were obtained from Thermo Fisher Scientific (Kanagawa, Japan). The sequences of the sense strand of each siRNA were as follows:

NCsiRNA:

5’-UUCUCUCACGCGCAGUACAUUU-3’
IL-1βsiRNA-1:
5’-GCUCUCCACCUAAUGGCAGAAA-3’
IL-1βsiRNA-2:
5’-GAGCAAAUGGAGUUGAGUCUGCA-3’

To evaluate the function of IL-1βsiRNA in the inhibition of endogenous IL-1β expression, IL-1βsiRNA-1 and IL-1βsiRNA-2 were transfected into RAW264.7 cells using LipofectamineRNAiMAX (Thermo Fisher Scientific Inc., Kanagawa, Japan), according to the protocol provided by the manufacturer. RAW264.7 cells were transfected with 10 nmol/L of siRNAs and harvested for RT-PCR analysis 48 h later. AteloGene (Koken, Tokyo, Japan) was used to transfer siRNAs into the murine carotid artery according to the manufacturer’s instruction. A mixture of siRNA solution and AteloGene (0.4 nmol/L siRNA/10 μL) was infused beneath the polyethylene cuff (above the adventitial side of the carotid artery) following its placement. Following the gelation of AteloGene, the cervical incision was sutured.

Histology
The carotid arteries were isolated and fixed with 4% paraformaldehyde. Following paraffin embedding, cross sections (5 μm) were cut, deparaffinized, rehydrated, and stained with hematoxylin and eosin (HE). For immunohistochemical analysis, these sections were incubated with primary antibodies reactive to vascular endothelial cadherin (VE-Cad), IL-1β, and MMP-9. Antigens were visualized using ImmPRESS-Alkaline Phosphatase Polymer Reagent and the alkaline phosphatase substrate, VECTOR Red (both Vector Laboratories, Burlingame, CA, USA). Sections were counterstained with hematoxylin. The positively stained area was analyzed using ImageJ (National Institutes of Health, Washington, DC, USA) after conversion of the color of the stained and non-stained areas to black and white, respectively, using Photoshop (Adobe Systems Inc., San Jose, CA, USA).

Tube Formation Assay
Matrigel (Basement Membrane Matrix Growth Factor Reduced; Corning Inc., Bedford, MA, USA) was used to examine the effect of IL-1β on the tube formation of vascular endothelial cells (VECs). Cold Matrigel (100 μL each) was placed in the wells of 24-well plates and incubated at 37°C for 30 min until it solidified. HUVECs were seeded on Matrigel-coated wells at a density of 4×10^4 cells/well in DMEM containing 2% FBS in the presence and absence of human bFGF and human IL-1β. Cells were incubated at 37°C for 24 h, and tube formation was assessed by counting the number of branching points in each well.

Cell Migration Assay
Human umbilical vein endothelial cells were cultured in 24-well plates using HuMedia-EG2 until they reached confluency. Cell layers were mechanically scratched with a 200 μL pipette tip and the medium was replaced with DMEM containing 2% FBS in the presence and absence of human bFGF and human IL-1β. Photographs of the scratched area were taken at this point and 24 h later. The area of the remaining empty zone was measured using ImageJ. The ability of cells to migrate into the scratched area was assessed by comparing the empty region at 0 and 24 h after scratching.

Western Blot Analysis
Total protein content was extracted from cultured cells in a cell lysis buffer (50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 1% NP-40) containing 1% volume of protease inhibitor cocktail (Sigma-Aldrich, Tokyo, Japan). Western blot analysis was performed as previously described.*

Statistical Analysis
All values are expressed as the mean±standard error of the mean. Statistical analyses were performed using the analysis of variance followed by the Student-Newman-Keuls test. Differences with a P value of <0.05 were considered statistically significant.

Results
Cuff Placement Following Carotid Artery Ligation Induced IPH
Carotid artery ligation induced neointimal formation (Figure 1). However, ligation alone did not induce IPH.
IL-1β Is Implicated in Intraplaque Hemorrhages

We first confirmed that IL-1β siRNA-1 and IL-1β siRNA-2 inhibited endogenous IL-1β expression both in vitro and in vivo (Figure S1). We used IL-1β siRNA-1 plus IL-1β siRNA-2 (IL-1β siRNA) to inhibit endogenous IL-1β in vivo and examined their effects on IPH in the following experiments. When IL-1β siRNA was transferred to the carotid artery, IPH significantly decreased in the neointima compared with NCsiRNA (Figure 3), suggesting that endogenous IL-1β was implicated in the progression of IPH.

Neovessel Formation Was Conserved in the Neointima When Endogenous IL-1β Expression Was Blocked

Leaky neovessels have been implicated in the extravasation of red blood cells and IPH. Therefore, we examined VE-Cad staining in the carotid artery with ligation plus cuff placement. Unexpectedly, VE-Cad staining was barely detected in the neointima of the NCsiRNA-transferred mice. Therefore, we decided to examine the role of endogenous IL-1β expression in the progression of IPH.

When the cuff was placed around the carotid artery, IPH was frequently induced in more than 90% of the cuff-placed samples.

Cytokine Expression in the Carotid Artery

We examined cytokine expression in the carotid artery (Figure 2). RNA was extracted from the intact carotid artery harvested from 14-week-old ApoE KO mice (Control group), the carotid artery with ligation alone (Ligation group: 5 weeks after carotid artery ligation performed on 9-week-old ApoE KO mice; i.e., the mice were 14 weeks old when the carotid artery was harvested), the carotid artery with cuff placement alone (Cuff group: Cuff placement was performed on 14-week-old ApoE KO mice) and the carotid artery with ligation plus cuff placement (Ligation_Cuff group). Angiopoietin-1 expression significantly increased in the Ligation_Cuff group compared with the Control group. Angiopoietin-2 expression significantly increased in the Ligation and Ligation_Cuff groups compared with the Control group. IL-1β expression most strikingly increased in the Ligation_Cuff group compared with the Control group. The expression of AM, VEGF-A, and TNF-α did not significantly change among the 4 groups. Interleukin-6 expression significantly decreased in the Ligation, Cuff, and Ligation_Cuff groups compared with the Control group.
extravasation of red blood cells and IPH. In contrast, mature neovessel formation seemed to occur in the IL-1β/siRNA-transferred carotid artery wherein IPH did not occur. These results suggest that endogenous IL-1β was implicated in the suppression of physiological angiogenesis, formation of leaky neovessels, and subsequent IPH in our model.
IL-1β Inhibited Angiogenesis In Vitro

As previous results suggested that IL-1β inhibited physiological angiogenesis, we examined the effects of IL-1β on angiogenesis in vitro. We used two assay systems. One was a tube formation assay in which HUVECs were plated on Matrigel-coated plates, and the formation of tube structures in extracellular matrices was examined (Figure 5A). Another was a cell migration assay in which a HUVEC monolayer was scratched with a pipette tip, and the repairing capacity of HUVECs was examined (Figure 6A). When HUVECs cultured in HuMedia-EG2 medium were plated on Matrigel-coated plates, a complete ring structure was observed (Figure 5A). When HUVECs cultured in DMEM containing 2% FBS were plated on Matrigel-coated plates, a branching of cords was observed, even a complete ring structure was seldom seen. When HUVECs cultured in DMEM containing 2% FBS plus IL-1β were seeded on Matrigel-coated plates, even the branching of HUVEC cords was barely detected. We scarcely observed the branching of HUVEC cords when they were cultured in DMEM containing less than 1% FBS (data not shown). We also used bFGF, a proangiogenic factor, and examined the effect of IL-1β on bFGF-induced tube formation, as it was reported that IL-1β inhibited bFGF-induced angiogenesis.10 HUVECs cultured in DMEM containing 2% FBS plus bFGF demonstrated a branching of cords formed by the cells, although an entirely closed ring structure was seldom observed. IL-1β significantly inhibited the branching induced by bFGF in a dose-dependent manner (Figure 5B).

We then examined the wound healing capacity of HUVECs. We preliminarily tested the condition of this assay and found that HUVECs cultured in DMEM containing less than 1% FBS did not sufficiently survive for 24 h to evaluate their wound healing capacity (data not shown). When HUVECs were cultured in DMEM containing 2% FBS plus bFGF, they significantly migrated toward the scratched area and repaired the injured area compared with when HUVECs were cultured in DMEM containing 2% FBS alone (Figure 6A). IL-1β significantly inhibited bFGF-induced wound healing in a dose-dependent manner (Figure 6B). These results suggested that IL-1β inhibited angiogenesis induced by bFGF in vitro.

IL-1β Inhibited the bFGF-Induced Activation of Akt (Protein Kinase B) and eNOS (Endothelial Nitric Oxide Synthase)

Evidence suggests that Akt/eNOS-dependent pathways are implicated in angiogenesis.11–15 Therefore, we examined the effect of IL-1β on the bFGF-induced activation of Akt and eNOS. As bFGF-induced phosphorylation of Akt and eNOS reached a maximal level at 15 and 30 min, respectively, after the bFGF stimulation in HUVECs (data not shown), we examined the effect of IL-1β on bFGF-induced phosphorylation of Akt and eNOS at these time points. IL-1β significantly inhibited the bFGF-induced phosphor-
SUZUKI E et al.

**Figure 8.** Interleukin (IL)-1β is implicated in matrix metalloproteinase (MMP)-9 expression. (A) MMP-9 expression in the neointima of the carotid artery that was treated with negative control siRNA (NCsiRNA) or IL-1βsiRNA-1 plus IL-1βsiRNA-2 (IL-1βsiRNA). Experiments were performed in the same way as described in Figure 3. Consecutive sections were used for hematoxylin and eosin (HE), MMP-9, and negative control staining (NC; without incubation with an anti-MMP-9 antibody). Bars indicate 100 μm. “H” indicates the area of intraplaque hemorrhage (IPH). (B) Statistical analysis of MMP-9 positive area in the neointima. *P<0.01 vs. IL-1βsiRNA administration (n=4 per group). (C) Western blot analysis of MMP-9 expression. Macrophages derived from THP-1 cells by treatment with phorbol 12-myristate 13-acetate (PMA) were stimulated with 10 ng/mL IL-1β for the indicated time (Left). Macrophages were also stimulated with increasing doses of IL-1β for 8 h (Right). The expression of β-actin was examined as the internal control. (D) Statistical analysis of MMP-9 expression. *P<0.05 vs. basal level (n=4 per group).

Maturation of Akt and eNOS in a dose-dependent manner (Figure 7). To clarify the mechanism by which IL-1β inhibited the bFGF-induced phosphorylation of Akt and eNOS, we examined the effect of IL-1β on type 1 receptor for bFGF (FGFR1) expression, as it was reported that IL-1β inhibited the binding of bFGF on HUVECs. IL-1β significantly inhibited FGFR1 expression in HUVECs, suggesting that a mechanism by which IL-1β inhibited angiogenesis caused by bFGF was the suppression of FGFR1 expression, which resulted in the inhibition of the phosphorylation of Akt and eNOS induced by bFGF.

**Matrix Metalloproteinase-9 Expression Was Suppressed by the Blockade of Endogenous IL-1β Expression**

We also examined the effect of the blockade of endogenous IL-1β expression on MMP-9 expression, as MMP-9 has been reportedly implicated in IPH. The immunostaining of MMP-9 in the neointima was significantly suppressed in the IL-1βsiRNA-administered group compared with the NCsiRNA-administered group (Figure 8A,B). As macrophages are a primary source of MMP-9 production, we examined the effect of IL-1β on MMP-9 expression in THP-1 cells treated with phorbol 12-myristate 13-acetate (PMA) to induce differentiation of THP-1 cells to macrophages. IL-1β significantly stimulated MMP-9 expression in time- and dose-dependent manners (Figure 8C,D).

**Discussion**

In this study, we showed that the blockade of endogenous IL-1β inhibited IPH progression following carotid artery ligation and cuff placement. We also demonstrated that the suppression of IPH by the blocking of endogenous IL-1β was positively correlated with the conservation of neovessel formation in the neointima. In addition, we showed that IL-1β inhibited angiogenesis in vitro, at least partly, via the suppression of bFGF-induced angiogenesis. These results suggested that endogenous IL-1β was implicated in pathological angiogenesis with fragile and leaky vascular endothelium, which lead to the extravasation of red blood cells and IPH.

Evidence suggests that neoangiogenesis with leaky VECs is implicated in the pathogenesis of IPH. In addition, plaque angiogenesis is reportedly correlated with plaque instability. Therefore, we expected that neoangiogenesis increased in the plaque with IPH. However, the VE-Cad
positive area significantly decreased in the plaque with IPH (treated with NCsiRNA) compared with that without IPH (treated with IL-1βsiRNA) in our model. Although the reason for the unexpected results remains unclear, we speculate that it is mainly because of an acute occurrence of IPH in our model. In contrast to human specimens in which IPH and plaque instability progress relatively slowly, IPH rapidly occurred in our model (within 5 days after cuff placement). Endogenous IL-1β expression might increase robustly and quickly in our model, and physiological angiogenesis might be potently suppressed in vivo, similar to that observed in vitro (tube formation assay and cell migration assay). Therefore, it might be difficult to detect the tube structure of pathologically formed neovessels. It was also possible that the pro-inflammatory and anti-inflammatory cytokine induction profiles in the vessels differed between humans and our mouse specimens, which might contribute to the different morphology.

IL-1β is a potent pro-inflammatory cytokine that stimulates adhesion molecule expression on VECs and is frequently expressed in human atherosclerotic plaques. In addition, IL-1β deficiency decreases the severity of atherosclerosis in ApoE KO mice. Accumulated evidence suggests that IL-1β is implicated in corneal, choroidal, tumor, and endothelial progenitor cell angiogenesis. However, the role of IL-1β in angiogenesis observed in atherosclerotic plaque remains unclear. Cozzolino et al demonstrated that IL-1β inhibited the proliferation of HUVECs in vitro and suppressed bFGF-induced corneal angiogenesis in vivo. Mount et al reported that IL-1β inhibited the proliferation, tube formation capacity, and wound healing capacity of cardiac microvascular endothelial cells. They also demonstrated that IL-1β suppressed microvessel sprouting from aortic rings. Baluk et al showed that transgenic IL-1β overexpression in airways augmented lymphangiogenesis but not angiogenesis. These results are compatible with ours and suggest that IL-1β has an anti-angiogenic activity in some circumstances. In contrast, IL-1β stimulated VEC proliferation in a human microvascular endothelial cell line. However, a cell line of VECs with a modification of telomerase catalytic protein that was not primary culture cells was used in this study. Therefore, it is possible that these cells responded to IL-1β differently compared with primary culture cells. Although it remains unclear how the pro-angiogenic and anti-angiogenic activity of IL-1β is regulated, it seems to depend upon the experimental models used.

We demonstrated that MMP-9 expression increased in the plaque with IPH (NCsiRNA-treated mice) compared with that without IPH (IL-1βsiRNA-treated mice). We also showed IL-1β stimulated MMP-9 expression in macrophages. Several reports have demonstrated that MMP-9 overexpression is correlated with IPH. In addition, many studies have shown that IL-1β induced MMP-9 expression in a variety of cells. Therefore, it is possible that endogenous IL-1β stimulated MMP-9 expression and induced IPH via a proteolytic degradation of the basement membrane of VECs, resulting in the formation of leaky neovessels.

Using double KO mice in which both IL-1 receptor type I gene (IL1R) and ApoE were deleted, Alexander et al reported that the genetic blockade of IL-1 signaling led to plaque instability (i.e., decrease in plaque collagen content, decrease in smooth muscle cell (SMC) content, increase in macrophage content, and increase in IPH) compared with the control ApoE null mice (IL1R was intact). These results were contradicting their original hypothesis. Although it remains unclear why the blockade of IL-1β inhibited IPH in our study and the blocking of IL1R stimulated IPH in their study, there are several possibilities. First, IL-1β signaling was permanently blocked in their double KO mice, while endogenous IL-1β expression was transiently suppressed (for 5 days) in our model. A genetic deletion of the IL1R might result in a compensatory response by other pro-inflammatory cytokines such as TNF-α, as discussed in their paper. These pro-inflammatory cytokines might promote the tissue inflammation that lead to IPH. Second, we used nearly 14- to 15-week-old mice to analyze IPH, whereas approximately 36-week-old mice were used to analyze spontaneous IPH in their study. Therefore, IPH in a relatively early stage of atherosclerotic plaque was analyzed in our study, whereas IPH in the late stage of the plaque was analyzed in their study. The effect of IL-1β on the progression of IPH might differ, depending on the stages of atherosclerotic plaque. Third, we used the carotid artery to examine IPH, whereas they used the brachiocephalic artery to analyze spontaneous IPH. The mechanisms of the progression of atherosclerosis might differ, depending on the arteries examined. In fact, the authors demonstrated that the SMC content in the plaque decreased and the macrophage content increased in the brachiocephalic artery, whereas the SMC content increased and the macrophage content was unchanged at the aortic root in the double KO mice. Further studies will be required to elucidate how the pro-atherogenic and anti-atherogenic capacity of IL-1β is regulated.

Limitations of This Study

We used the carotid artery of ApoE KO mice with ligation and cuff placement to analyze IPH. This model is convenient because IPH can be detected reproducibly in a short period. However, IPH occurred rapidly in this model and the time-course of its progression seems to be different from spontaneous IPH. On the other hand, few experimental models exist in which spontaneous IPH can be analyzed reproducibly and conveniently. A novel animal model to investigate the pathogenesis of IPH will be required to elucidate the mechanisms of IPH and develop a strategy to prevent IPH.

Conclusions

Endogenous IL-1β seems to be implicated in the progression of IPH via the induction of leaky neovessels in the atherosclerotic plaque and MMP-9 expression. Modulation of IL-1β expression will be a promising strategy to prevent IPH and subsequent plaque rupture.

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