Critical Role of Bone Marrow Apoptosis-Associated Speck-Like Protein, an Inflammasome Adaptor Molecule, in Neointimal Formation After Vascular Injury in Mice

Noriyuki Yajima, MD*; Masafumi Takahashi, MD, PhD*; Hajime Morimoto, DVM; Yuji Shiba, MD, PhD; Yasuko Takahashi, MD; Junya Masumoto, MD, PhD; Hiroyiko Ise, PhD; Junji Sagara, PhD; Jun Nakayama, MD, PhD; Shun’ichiro Taniguchi, PhD; Uichi Ikeda, MD, PhD

Background—Inflammatory cytokines such as interleukin (IL)-1β and IL-18 play an important role in the development of atherosclerosis and restenosis. Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) is an adaptor protein that regulates caspase-1–dependent IL-1β and IL-18 generation; however, the role of ASC in vascular injury remains undefined. Here, we investigated the contribution of ASC to neointimal formation after vascular injury in ASC-deficient (ASC−/−) mice.

Methods and Results—Wire-mediated vascular injury was produced in the femoral artery of ASC−/− and wild-type mice. Immunohistochemical analysis revealed that ASC was markedly expressed at the site of vascular injury. Neointimal formation was significantly attenuated in ASC−/− mice after injury. IL-1β and IL-18 were expressed in the neointimal lesion in wild-type mice but showed decreased expression in the lesion of ASC−/− mice. To investigate the contribution of bone marrow–derived cells, we developed bone marrow–transplanted mice and found that neointimal formation was significantly decreased in wild-type mice in which bone marrow was replaced with ASC−/− bone marrow cells. Furthermore, in vitro experiments showed that the proliferation activity of ASC−/− vascular smooth muscle cells was not impaired.

Conclusions—These findings suggest that bone marrow–derived ASC is critical for neointimal formation after vascular injury and identify ASC as a novel therapeutic target for atherosclerosis and restenosis. (Circulation. 2008;117:3079-3087.)

Key Words: angioplasty ■ bone marrow cell ■ cytokine ■ inflammation ■ restenosis

Neointimal formation after vascular injury is the pathologic basis of atherosclerosis and restenosis after percutaneous coronary intervention such as angioplasty and stenting. Although the pathogenic mechanisms have not been completely resolved, an accumulating body of evidence suggests that inflammatory response plays a key role in these processes. Interleukin (IL)-1β and IL-18, inflammatory cytokines that mediate a wide range of immune and inflammatory responses,1 have been shown to be involved in the development of restenosis and atherosclerosis.2–4 Generation of mature IL-1β and IL-18 requires proteolytic processing of IL-1β and IL-18 precursors by the converting enzyme caspase-1 (also known as IL-1-β-converting enzyme).1 Recent studies have suggested that caspase-1 is activated within a multiple adaptor complex called the inflammasome complex.5 Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), also known as target of methylation-induced silencing-1,6 is a putative component of the inflammasome complex. We originally identified ASC as an intracellular protein that generates speck-like aggregations in apoptotic HL-60 cells treated with chemotherapeutic agents.7 ASC is a 21.5-kDa cytosolic protein that carries a caspase recruitment domain at the C-terminal and a domain (PYD) homologous to pyrin, a gene product responsible for familial Mediterranean fever, which is an inherited systemic disease characterized by recurrent episodes of fever and inflammation, at the N-terminal.8 ASC serves as a molecular bridge between PYD and caspase recruitment domain–containing signaling molecules. In addition, we and other investigators recently demonstrated that ASC mediates the recruitment and activation of caspase-1, thereby regulating caspase-1–mediated maturation of IL-1β and IL-18.9,10 Therefore, we hypothesized that ASC can regulate inflammation and subsequent neointimal formation at the site of vascular injury.

Received June 12, 2007; accepted March 31, 2008.
From the Departments of Cardiovascular Medicine (N.Y., M.T., H.M., Y.S., H.I., U.I.) and Molecular Oncology (Y.T., J.S., S.T.), Shinshu University Graduate School of Medicine, and Department of Pathology (J.M., J.N.), Shinshu University School of Medicine, Matsumoto, Japan.
*The first 2 authors contributed equally to this work.
The online Data Supplement can be found with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.107.746453/DC1.
Correspondence to Masafumi Takahashi, MD, PhD, Department of Cardiovascular Medicine, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. E-mail masafumi@shinshu-u.ac.jp
© 2008 American Heart Association, Inc.
Circulation is available at http://circ.ahajournals.org
DOI: 10.1161/CIRCULATIONAHA.107.746453
Determination of Apoptosis

Apoptotic cells were identified by the terminal transferase-mediated dUTP nick-end labeling (TUNEL) staining kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions.

Cell Cultures

Bone marrow cells were collected from wild-type and ASC−/− mice and analyzed by flow cytometry and Western blotting. Murine vascular smooth muscle cells (VSMCs) were isolated from the aorta of 4- to 6-week-old wild-type and ASC−/− mice and cultured in DMEM (Sigma, St Louis, Mo) supplemented with 10% FBS (Hyclone, Logan, Utah).13 VSMCs with 3 to 6 passages were used in the experiments.

Flow Cytometry Analysis

Blood samples were collected from the mice at baseline and 48 hours after vascular injury. Circulating cells were identified using a nucleated cell fraction. The cells were double labeled with FITC-conjugated anti-CD34 (clone RAM34, BD Biosciences, San Jose, Calif) and PE-conjugated anti–Fik-1 (VEGFR2/KDR: clone Avas12x1, BD Biosciences) antibodies and examined by flow cytometry. To identify ASC expression in blood cells, peripheral blood cells were collected and permeabilized with an intracellular cytometry. To identify ASC expression in blood cells, peripheral blood cells were collected and permeabilized with an intracellular staining kit (Roche Diagnostics, Mannheim, Germany).

Materials and Methods

Animals

The animal experimental protocol used in this study was reviewed and approved by the Shinshu University Guide for Laboratory Animals, which conforms to the National Institutes of Health (NIH) guidelines. ASC−/− mice were generated as described previously9 and backcrossed with the C57BL/6 strain mice. The resulting littermates were used for this study (wild-type: ASC+/+ littermates and homozygous ASC−/− mice, 8 to 12 weeks old). Other wild-type mice (C57BL/6, male, 8 to 12 weeks old) were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were fed a standard diet and water and maintained on a 12-hour light/dark cycle.

Wire-Mediated Vascular Injury

Wire-mediated vascular injury of the right femoral artery was produced as described previously by Sata et al.11 We confirmed that this procedure induced reproducible neointimal formation in 8- to 12-week-old C57BL/6 mice.12

BMT Mice

BMT mice were produced as described previously.12 Whole bone marrow cells from wild-type and ASC−/− mice were harvested by flushing their femurs with PBS. Red blood cells were lysed with ACK buffer (150 mmol/L NH4Cl, 10 mmol/L KHCO3, 0.1 mmol/L EDTA, pH 7.2) at 4°C for 20 minutes. The cells were washed 3 times with PBS and resuspended in 0.5 mL PBS. Recipient mice (wild-type and ASC−/− mice, 6 to 8 weeks old) were lethally irradiated with a total dose of 9 Gy (MBR-155R2, Hitachi, Japan) and injected with bone marrow cells through the tail vein. To verify the reconstitution of bone marrow after transplantation by this protocol, we used green fluorescent protein–transgenic mice (C57BL/6 background, kindly provided by Professor M. Okabe, Osaka, Japan) as donors. Flow cytometry analysis revealed that at 6 weeks after transplantation, peripheral blood cells consisted of >90% green fluorescent protein–positive cells (Figure I of the online Data Supplement). Using this protocol, we produced 3 types of BMT mice: wild-type to wild-type (BMTwild→wild) mice, wild-type to ASC−/− mice (BMTwild→ASC−/−), and ASC−/− to wild-type (BMTASC−/−→wild) mice.

Histology and Immunohistochemistry

Histology and immunohistochemistry were performed as described previously.13 Details are given in the online Data Supplement. We confirmed the detection of reendothelialization after injury by using antibodies against CD31 and VE-cadherin (supplemental Figure II).

In Situ Hybridization

A digoxigenin-labeled RNA probe was prepared, and in situ hybridization was performed as described previously.14 Details are given in the online Data Supplement.
that the expression of ASC was clearly observed in the injured site at 7 days and in the neointimal lesion at 21 days after injury (Figure 1A). In contrast, we observed expression of ASC in the endothelium of the intact artery. To identify the cells that express ASC in the neointima, we performed double immunofluorescence staining using antibodies against ASC and macrophages (F4/80) or VSMCs (α-SMA) and found that ASC was colocalized with macrophages or VSMCs (Figure 1B). Furthermore, in situ hybridization revealed the expression of ASC mRNA in the vascular wall after injury (Figure 1C).

**Effect of ASC Deficiency on Neointimal Formation**

Because we previously demonstrated that neointimal formation is completed at 21 days after injury,12,13 we evaluated the effect of ASC deficiency on neointimal formation at 21 days after vascular injury. Hematoxylin and eosin and elastica–van Gieson staining revealed that the neointimal formation was

![Figure 1. ASC expression after vascular injury. Wire-mediated vascular injury was produced in wild-type mice. The injured and uninjured (intact) femoral arteries were excised at 7 and 21 days after injury. A, Immunohistochemical staining for ASC was performed. Rabbit IgG was used as a negative control. Representative photographs are shown (n=3). Bar represents 50 μm. B, Double-immunofluorescence staining for ASC and macrophages (F4/80) or VSMCs (α-SMA) was performed. Nucleic acid was stained with DAPI. Bar represents 10 μm. C, In situ hybridization for ASC mRNA was performed. Bar represents 100 μm.](http://circ.ahajournals.org/)

![Figure 2. Effect of ASC deficiency on neointimal formation. Wire-mediated vascular injury was produced in wild-type (WT; n=11) and ASC−/− (n=12) mice. The femoral arteries were excised at 21 days after injury. Sample sections were stained with hematoxylin and eosin (HE) and elastica–van Gieson (EVG), and neointimal formation was evaluated. A, Representative photographs of hematoxylin and eosin and elastica–van Gieson staining. Arrowheads indicate the internal elastic lamina. Bar represents 100 μm. B, C, Bar graphs show the neointimal area (B), medial area (C), and intima-to-media ratio (D) quantified by NIH Image software. Data are expressed as mean±SEM. **P<0.01.](http://circ.ahajournals.org/)

![Figure 3. IL-1β and IL-18 expression and apoptosis. Wire-mediated vascular injury was produced in wild-type (WT) and ASC−/− mice. The femoral arteries were excised at 2 hours (for TUNEL staining) and 21 days (for IL-1β and IL-18 staining) after injury. A, Immunohistochemical staining for IL-1β and IL-18 was performed. Representative photographs are shown. Rabbit IgG was used as a negative control. B, TUNEL staining was performed. C, The number of TUNEL-positive cells was quantified. Data are expressed as mean±SEM (each n=6). Bar represents 100 μm.](http://circ.ahajournals.org/)
markedly reduced in ASC−/− mice compared with wild-type mice (Figure 2A). Quantitative analysis showed that the neointimal area and the intima-to-media ratio were significantly reduced in ASC−/− mice (P=0.002 and P=0.007, respectively; Figure 2B and 2D). However, no significant difference was observed in the medial area between wild-type and ASC−/− mice (P=0.300; Figure 2C).

IL-1β and IL-18 Expression and Apoptosis
Because ASC regulates the maturation of IL-1β and IL-18,5,9 we performed immunohistochemical analysis to detect IL-1β and IL-18 in the injured arteries. As shown in Figure 3A, IL-1β and IL-18 were expressed in the neointimal lesions of wild-type mice but showed decreased expression in the lesions of ASC−/− mice.

To assess the involvement of apoptosis after vascular injury in wild-type and ASC−/− mice, TUNEL staining was performed. Consistent with a previous report,11 a substantial number of TUNEL-positive cells were detected in the vascular wall at 2 hours after injury in wild-type and ASC−/− mice (Figure 3B). Quantitative analysis showed no significant difference in TUNEL-positive cells between these mice. Furthermore, the expression of activated caspase-3 also was detected in the vascular wall after injury of both wild-type and ASC−/− mice (supplemental Figure III).

Detection of Endothelial Cells, Macrophages, and VSMCs
Because we previously demonstrated that early reendothelialization after vascular injury results in attenuation of neointimal formation,12 immunohistochemical analysis of the endothelial marker CD31 and VE-cadherin was performed. No significant difference was observed in reendothelialization at 7 days after injury between wild-type and ASC−/− mice (Figure 4A and 4B). Flow cytometry analysis also showed no difference in the number of peripheral CD34+/Flk-1+ cells (ordinary endothelial progenitor cell marker12,17) at 24 hours after vascular injury between wild-type and ASC−/− mice (wild-type, 0.015±0.005%; ASC−/−, 0.007±0.003%; P=NS). We further performed immunohistochemical analysis to detect macrophages (F4/80) and VSMCs (α-SMA) and assessed the cellular contents of neointima in wild-type and ASC−/− mice. Consistent with previous reports,11,13 the neointimal lesion was composed of substantial number of VSMCs and some macrophages (Figure 4C through 4F). Although the total number of VSMCs and macrophages in the neointima of ASC−/− mice tended to be lower than that in the neointima of wild-type mice (Figure 4C and 4E), the number of VSMCs and macrophages per unit of the neointimal area did not differ between wild-type and ASC−/− mice (Figure 4D and 4F).

Contribution of Bone Marrow–Derived Cells
To determine the contribution of bone marrow–derived cells to the attenuation of neointimal formation after vascular injury, we produced 3 types of BMT mice (BMTWild→WT mice, BMTWild→ASC−/− mice, and BMTASC−/−→WT mice) and evaluated neointimal formation after injury. The forma-
tion of neointima after vascular injury in BMT\textsuperscript{Wild}$\rightarrow$Wild mice was similar to that in wild-type mice, and it tended to be reduced in BMT\textsuperscript{Wild}$\rightarrow$ASC$^{-/-}$ mice (Figure 5). Importantly, neointimal formation in BMT\textsuperscript{ASC$^{-/-}$$\rightarrow$Wild mice was markedly reduced compared with that in BMT\textsuperscript{ASC$^{-/-}$}ASC$^{-/-}$ mice \((P=0.0003)\) and BMT\textsuperscript{ASC$^{-/-}$}Wild mice \((P=0.015)\). These results indicate that bone marrow–derived ASC is critical for neointimal formation after injury. We also examined whether bone marrow–derived cells contribute to the neointimal lesion in BMT mice in which bone marrow was replaced with that of green fluorescent protein–transgenic mice and detected some green fluorescent protein–positive cells among the \(\alpha\)-SMA–positive cells \((11.2\pm2.8\%)\) in the neointimal lesion after injury (supplemental Figure IV).

**ASC Expression in Bone Marrow–Derived Cells**

To determine the types of bone marrow–derived blood cells that express ASC, peripheral nuclear cells were analyzed by flow cytometry. The expression of ASC was detected in CD3$^+$ cells (T cells), Mac-1$^-$/Gr-1$^+$ cells (monocytes), and Gr-1$^+$ cells (granulocytes) (Figure 6A through 6C). In particular, the expression of ASC in T cells showed 2 peaks of high and low expression. We further examined whether ASC-expressing cells were affected by the vascular injury and found that the injury had no significant effect on the percentage of ASC-expressing cells \((day 1: CD3^+ cells, 55.4\pm13.4\% \text{ versus } 48.2\pm12.1\%; \text{Mac-1}^-/\text{Gr-1}^- \text{cells, } 31.5\pm6.7\% \text{ versus } 23.1\pm10.4\%; \text{Gr-1}^+ \text{cells, } 24.0\pm4.7\% \text{ versus } 12.4\pm5.4\%; \text{P=NS})\). Western blot analysis also confirmed ASC expression in bone marrow cells isolated from wild-type mice but not in those from ASC$^{-/-}$ mice (Figure 6D).

**Proliferation Activity of VSMCs**

Neointimal lesion after vascular injury mainly comprises proliferative VSMCs.\textsuperscript{11} Because the mitogen-activated protein kinase pathway is thought to be critical for the VSMC proliferation signal,\textsuperscript{18} the effect of serum or growth factor on ERK1/2 and p38 activation in cultured VSMCs isolated from ASC$^{-/-}$ mice was investigated. Western blot analysis revealed marked phosphorylation of ERK1/2 and p38 in response to FBS and PDGF-BB in wild-type–derived VSMCs (Figure 7A and 7B). The PDGF-BB–induced phosphorylation of ERK1/2 and p38 reached a peak at 5 minutes and subsequently declined. In the ASC$^{-/-}$–derived VSMCs, a similar phosphorylation pattern of ERK1/2 and p38 was observed. We further examined the proliferation activity of cultured VSMCs isolated from ASC$^{-/-}$ mice by using the BrdU incorporation assay. Treatment with PDGF-BB but not IL-1$\beta$ and IL-18 stimulated proliferation in both wild-type–derived and ASC$^{-/-}$–derived VSMCs, and no significant difference was observed in the activity between these VSMCs (Figure 7C). To determine cell proliferation activity in vivo, we performed immunohistochemical staining for proliferating cell nuclear antigen and found that the number of

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Contribution of bone marrow–derived ASC to neointimal formation. BMT\textsuperscript{Wild}$\rightarrow$Wild mice \((n=6)\), BMT\textsuperscript{Wild}$\rightarrow$ASC$^{-/-}$ mice \((n=10)\), and BMT\textsuperscript{ASC$^{-/-}$$\rightarrow$Wild mice \((n=11)\) were developed, and wire-mediated vascular injury was produced in them 8 weeks after BMT. The femoral arteries were excised at 21 days after injury. The sample sections were stained with hematoxylin and eosin (HE) and elastica–van Gieson (EVG), and neointimal formation was evaluated. A, Representative photographs of hematoxylin and eosin and elastica–van Gieson staining. Bar represents 100 \(\mu\)m. B, C, Bar graphs show the neointimal area (B), medial area (C), and intima-to-media ratio (D) quantified by NIH Image software. Data are expressed as mean\(\pm\)SEM. WT indicates wild type. *\(P<0.05\), **\(P<0.01\).
proliferating cell nuclear antigen–positive cells decreased in the neointimal lesion of ASC−/− mice (Figure 7D).

Discussion

The major findings of this study are as follows: (1) ASC was markedly expressed at the site of vascular injury; (2) ASC deficiency reduced the expression of IL-1β and IL-18 in the neointimal lesion and attenuated neointimal formation after vascular injury; (3) the absence of ASC specifically in bone marrow cells reduced neointimal formation; and (4) the proliferation activity of ASC-deficient VSMCs was not impaired in in vitro experiments. These findings suggest that bone marrow–derived ASC plays a critical role in neointimal formation after vascular injury.

ASC is an adaptor molecule that mediates inflammatory and apoptotic signals. Recent investigations suggest that the PYD domain of ASC functions as the signal transduction/oligomerization domain, whereas the caspase recruitment domain functions as the effector domain for activation of caspase-1, thereby regulating the expression of IL-1β and IL-18.6 Accumulating reports have demonstrated the important role of ASC in the fields of immunology and oncology5,6,10,19,20; however, to date, no information is available on the role of ASC in cardiovascular diseases. In the present study, we clearly demonstrated that neointimal formation and expression of IL-1β and IL-18 after vascular injury were attenuated in ASC−/− mice. Furthermore, we recently observed the expression of ASC in human atherosclerotic lesions (M. Takahashi and J. Nakayama, unpublished observation, 2008); this suggests the potential role of ASC and its downstream cytokines in neointimal formation. Indeed, several lines of evidence indicate that IL-1β and IL-18 play an important role in the pathogenesis of atherosclerosis and restenosis after percutaneous coronary intervention. For instance, increased expression of IL-1β and IL-18 has been reported in human atherosclerotic plaques.4,21 Genetic correlations between IL-1 genotype and the risk of restenosis after percutaneous coronary intervention have been reported.22 Moreover, recent studies have shown a strong correlation between higher IL-18 plasma levels and restenosis after percutaneous coronary intervention23 or clinical outcome in patients with coronary artery disease.24 Experimental animal models of other vascular injuries also demonstrated the critical role of IL-1β and IL-18 in neointimal formation. Isoda et al2 reported that neointimal formation is attenuated by the inhibition of the IL-1 signaling pathway in a murine model of perivascular cuff-induced vascular injury. Maffia et al3 also showed that IL-18 expression was increased in carotid arteries after balloon injury in rats and that neutralization of IL-18 inhibited neointimal formation. Thus, these results indicate that IL-1β and IL-18 act as important mediators for the development of atherosclerosis and restenosis. Interestingly, the recent AtheroGene study showed that plasma caspase-1 levels are predictive of future cardiovascular death in patients with coronary artery disease.25 Therefore, we postulated that ASC may regulate the expression of IL-1β and IL-18 via a caspase-1–dependent pathway in the vascular wall after vascular injury and modulate vascular inflammation and VSMC proliferation.

It is also noted that ASC deficiency had no effect on apoptosis after vascular injury. Although it has recently been accepted that ASC regulates the process of apoptosis, its precise mechanisms are unknown. Evidence from overexpression studies indicates that ASC can promote apoptosis in a Bax- and caspase–9–dependent manner,20,26 and antisense-mediated knockdown of ASC protects the cells from apoptosis induced by cytotoxic agents.7 ASC also has been implicated in the apoptotic process induced by the death receptor.

Figure 6. ASC expression in bone marrow–derived cells. A–C, Peripheral blood cells were isolated from wild-type and ASC−/− mice. Expression of ASC in CD3+ cells (T cells), Mac-1+/Gr-1− cells (monocytes), or Gr-1+ (granulocytes) was analyzed by flow cytometry. Results are representative of 3 independent experiments. D, Bone marrow cells were isolated from wild-type and ASC−/− mice. Cell lysates were prepared and analyzed by Western blotting with antibodies against ASC or β-actin (n=4 for each).
Although we observed no significant difference in vascular wall apoptosis in response to injury between wild-type and ASC−/− mice, further investigations are required to elucidate the role of ASC in apoptosis after injury.

Increasing evidence indicates the importance of vascular progenitor cells derived from bone marrow in vascular development and remodeling. In particular, we and other investigators have shown that reendothelialization (eg, vascular repair) by bone marrow–derived endothelial progenitor cells is one of the important determinant factors for neointimal formation after vascular injury.12,27,28 In this study, however, ASC deficiency had no effect on reendothelialization at the early phase after vascular injury. Furthermore, ASC deficiency did not influence the number of endothelial progenitor cells in the peripheral circulation; this suggests that inhibition of neointimal formation in ASC−/− mice may be mediated through mechanisms different from those of vascular repair by reendothelialization. Furthermore, it is unlikely that reendothelialization after vascular injury is influenced by the ASC-regulated inflammatory cytokines IL-1β and IL-18.

We demonstrated that ASC deficiency in bone marrow cells reduced neointimal formation after vascular injury. Recent investigations have shown that bone marrow cells participate in neointimal formation after vascular injury29; however, the role of bone marrow cells is not yet fully understood. ASC has been shown to regulate caspase-1–mediated IL-1β and IL-18 production.9,10 Furthermore, ASC is reported to possess the potential to modulate nuclear factor-κB activation.30 Therefore, ASC may regulate inflammatory cytokine production through caspase-1–dependent and –independent ways. Interestingly, ASC-deficient VSMCs did not display any overt defects in proliferation activity and mitogen-activated protein kinase activation. These data are consistent with the results of BMT experiments that neointimal formation was not effectively reduced in ASC−/− mice in which bone marrow was replaced with wild-type bone marrow cells. Taken together, these results suggest a critical role of bone marrow–derived ASC in neointimal formation after vascular injury.

In the present study, we used a wire-mediated vascular injury model because it allows us to reproduce complete endothelial cell denudation and neointimal formation after injury.11,12 This model induces robust neointimal formation at 21 days after injury even in the control mice. Although the contribution of bone marrow cells to neointimal formation is controversial, Tanaka et al29 reported that wire-mediated vascular injury is suitable for investigating the role of bone marrow cells in vascular remodeling after injury. We also
detected the bone marrow–derived α-SMA–positive cells in the neointima. Furthermore, there are some reports in the literature describing that bone marrow–derived cells potentially participate in the lesion formation after injury.31–34 Recently, Yamada et al32 demonstrated that bone marrow–derived α-SMA–positive cells did not express SM1 (a marker for relatively mature VSMCs), suggesting that these cells in the neointima have a relatively immature phenotype.

The present study has several limitations. First, the model used is not a reliable experimental model of human angioplasty because the injury was produced on a normal nonatheromatous artery. Second, the femoral artery is not similar to other arteries (eg, coronary artery) with respect to its response to vascular injury. Therefore, further investigations are required to elucidate the precise role of ASC in the development of atherosclerosis and restenosis.

Conclusions

We clearly demonstrated that ASC deficiency reduced neointimal formation after vascular injury. In particular, the absence of ASC in bone marrow cells plays a critical role in the attenuation of the progression of neointimal formation. Our data suggest that bone marrow–derived ASC is essential for the development of atherosclerosis and restenosis after percutaneous coronary intervention and identify ASC as a novel therapeutic target for cardiovascular diseases.

Acknowledgments

We thank Junko Nakayama, Tomoko Hamaji, and Kazuko Misawa for excellent technical assistance.

Sources of Funding

This study was supported by research grants from the Ministry of Education, Culture, Sports, Science and Technology (to Dr M. Takahashi); the Ministry of Health, Labor and Welfare (to Drs M. Takahashi and Ikeda); and a Grant-in-Aid for Scientific Research (C-18590524 to Dr Masumoto) from the Japanese Society for the Promotion of Science.

Disclosures

None.

References

CLINICAL PERSPECTIVE

Accumulating reports have demonstrated an important role of the inflammation-induced adaptor complex, called the inflammasome, in the fields of immunology and oncology. To date, there has been no report describing the role of inflammasome in cardiovascular diseases. Apoptosis-associated speck-like protein (ASC) containing a caspase recruitment domain is an adaptor protein that forms inflammasome and regulates caspase-1–dependent interleukin (IL)-1β and IL-18 generation. Here, we show that ASC is markedly expressed in the site of vascular injury in mice and colocalized with macrophages or vascular smooth muscle cells. Neointimal formation after vascular injury is significantly attenuated in ASC-deficient mice compared with that in wild-type mice. The expression of IL-1β and IL-18 was observed in the neointimal lesion of wild-type mice but showed decreased expression in the lesion of ASC-deficient mice. Additional studies showed that ASC deficiency influences neointimal formation by regulating the behavior of cells that are derived from bone marrow. Our results show that bone marrow–derived ASC is critical for neointimal formation after vascular injury and identify ASC as a potential therapeutic target for atherosclerosis and restenosis after percutaneous coronary intervention.
Critical Role of Bone Marrow Apoptosis-Associated Speck-Like Protein, an Inflammasome Adaptor Molecule, in Neointimal Formation After Vascular Injury in Mice
Noriyuki Yajima, Masafumi Takahashi, Hajime Morimoto, Yuji Shiba, Yasuko Takahashi, Junya Masumoto, Hirohiko Ise, Junji Sagara, Jun Nakayama, Shun’ichiro Taniguchi and Uichi Ikeda

Circulation. 2008;117:3079-3087; originally published online June 9, 2008;
doi: 10.1161/CIRCULATIONAHA.107.746453
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/24/3079

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2008/06/12/CIRCULATIONAHA.107.746453.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/