Mini Review

Chronic inflammation in intracranial aneurysm formation

Tomohiro Aoki[1, 2, *], Miyuki Fukuda[2, 3] and Shuh Narumiya[2]

[1]Innovation Center for Immunoregulation Technologies and Drugs (AK project), Kyoto University Graduate School of Medicine, Kyoto, Japan
[2]Core Research for Evolutional Science and Technology (CREST), Kyoto University Graduate School of Medicine, Kyoto, Japan
[3]Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

Intracranial aneurysm (IA) is a cerebrovascular disease usually affected the bifurcation sites of intracranial arteries. IA is socially important because IA can develop a lethal subarachnoid hemorrhage after rupture. However, today, there is no medical treatment for IAs because of the lack of knowledge regarding the mechanisms regulating IA formation. Experimental analyses using human IA specimen have revealed the presence of long-lasting inflammation in IA lesions. In other point of view, from studies of flow dynamics, IA formation is presumed to be triggered under high wall shear stress loaded on arterial bifurcations. Nonetheless, the mechanisms regarding how IA formation is regulated under high wall shear stress and how the inflammation lasts for a long period remain to be elucidated. Recently, experimental studies using rodent IA models have revealed a critical role of prostaglandin (PG) E_2 signaling to IA formation. In brief, COX-2, a PGE_2-producing enzyme, is induced under high wall shear stress in endothelial cells. COX-2-producing PGE_2, then, activates NF-κB, a critical transcription factor for IA formation, via EP2. Because NF-κB transcriptionally induces COX-2, a positive feedback loop consisting of COX-2-PGE_2-EP2-NF-κB signaling is formed and high wall shear stress induced inflammation becomes chronic. Here, as a result of NF-κB-mediated MCP-1 induction, macrophages infiltrate in IA walls and expand inflammation into whole arterial walls beyond endothelial cells via secreting a large amount of pro-inflammatory molecules. In summary, the presence of positive feedback loop and macrophage infiltration are two key events regulating the chronicity of inflammation contributing to IA formation.

Rec.8/29/2013, Acc.9/10/2013, pp283-287

* Correspondence should be addressed to:
Tomohiro Aoki, Innovation Center for Immunoregulation Technologies and Drugs (AK project), Konoe-cho Yoshida, Sakyo-ku, Kyoto City, Kyoto 606-8315, Japan. Phone: +81-75-753-9511, Fax: +81-75-753-9500, E-mail; tomoaoki@kuhp.kyoto-u.ac.jp

Key words chronic inflammation, intracranial aneurysm, macrophage, NF-κB, prostaglandin

Social importance and current problem of intracranial aneurysm treatment

Intracranial aneurysm (IA) is a regional bulging of intracranial arterial walls and usually affects bifurcation sites of intracranial arteries. IA is a popular disease among the general public and can be detected about 1 to 5% of general

population[1]. Social importance of IA depends on a resultant subarachnoid hemorrhage after rupture, because IA itself is an asymptomatic lesion before rupture in most every case. Subarachnoid hemorrhage is a devastating disease with quite a high mortality rate, which reaches 30 to 50 %, and also a high morbidity rate despite the intensive treatment and recent advancement of therapeutic devices[2]. Treatment to prevent the rupture of pre-existing IA is, therefore, socially demanded. Today, IA is treated only by surgical procedures, microsurgical clipping and endovascular coiling[3]. These surgical manipulations have greatly developed and certainly provide the certain level of safety. However, considered with the nature of IA as an asymptomatic lesion before rupture, there is a considerable rate of complications such as an ischemic complication or brain damage[3]. Furthermore, there is no therapeutic strategy available for patients without surgical indications, such as patients with small IAs or with a high estimated risk of surgical procedures. Current problem of IA treatment is, therefore, the lack of less invasive medical treatment to prevent a rupture of IA and a resultant subarachnoid hemorrhage. To overcome the current situation, the mechanisms regulating IA formation and rupture need to be revealed and therapeutic targets for IA treatment should be identified.

Involvement of inflammation in intracranial aneurysm of human

Various analyses using human specimen have clarified the presence of inflammation in IA lesions and proposed the notion that active inflammatory responses contribute to the pathogenesis of IA[4, 5]. For example, in histopathological analyses, infiltration of inflammatory cells, especially most abundantly macrophages, is demonstrated[6, 7]. Furthermore, in comprehensive gene expression analyses, the induction of pro-inflammatory genes, such as TNF-α , in IA lesions is identified and, through subsequent bioinformatics analyses, the activation of inflammation-related cascades is extracted[8-10]. Epidemiologically, patients with sepsis or autoimmune-vasculitis are more likely to develop IAs[1].

In other point of view, hemodynamic force, especially high wall shear stress, has been recognized as a trigger of IA formation through the studies regarding hemodynamics in IA lesions[11-13]. For example, at the neck portion of IA where IA formation initiates or at the prospective site of IA formation in intracranial arteries, usually a bifurcation site, wall shear stress is much higher than surrounding arterial walls[11-13]. However, how high wall shear stress regulates IA formation remains to be elucidated.

Unfortunately, however, because of the considerable limitations related with experiments using human specimen, we cannot fully understand the underlying mechanisms of IA formation, for example how high wall shear stress induces inflammation and regulates IA formation or how inflammation becomes chronic. To clarify the mechanisms regulating IA formation, therefore, animal models of IA have been developed[14-16]. These animal models, indeed, greatly contribute to the clarification of some of the regulatory mechanisms of IA formation as discussed in following section.

Chronic inflammation and intracranial aneurysm formation

Experimental findings achieved from animal models of IA have clarified the critical role of chronic inflammation in the pathogenesis of IA formation[5, 17]. In these animal models, IAs are induced by the increase of hemodynamic stress on contralateral arterial bifurcations of intracranial arteries through hemi-carotid ligation and salt overloading without direct handling of intracranial arterial walls[14, 16]. Because IAs are spontaneously induced by the hemodynamic force like human IAs and IAs induced in animals share the similar histopathological findings with human IAs such as the disruption of the internal elastic lamina and the degenerative change of media, these animal models of IA are considered as appropriate models for human IAs[14, 16]. Using these animal models, contribution of pro-inflammatory molecules, such as NF-κB[18], IL-1β[19], Matrix Metalloprotainase-9 (MMP-9)[20], inducible nitric oxide synthase (iNOS)[21] and monocyte chemoattractant protein-1 (MCP-1)[22, 23] to IA formation has been revealed supporting the notion that IA is a chronic inflammatory disease of intracranial arterial walls as proposed from human studies[5]. Here, because NF-κB regulates the induction of a wide range of pro-inflammatory genes, including genes involved in the pathogenesis of IA, as a critical transcription factor in inflammatory settings, we have focused on NF-κB as a critical mediator of IA formation. Indeed, all the NF-κB deficiency in mice[18], the inhibition of transcriptional activity by decoy oligonucleotides[18] and the pharmacological inhibition by some compounds in rats[24, 25] significantly suppressed IA formation through inhibiting inflammatory responses in IA walls.

As previously discussed, high wall shear stress seems to greatly influence IA formation as an inducer revealed by analy-

ses of fluid dynamics. Consistent with these presumptions, IA formation in animal models, histologically characterized as the disrupted internal elastic lamina and degenerative change of media, initiates at the site loaded on high wall shear stress and high walls shear stress gradient[26, 27]. Furthermore, induction of pro-inflammatory molecules, which regulates IA formation, can be detected at the region loaded on high wall shear stress[28] suggesting that high wall shear stress triggers inflammation resulting in IA formation. We have recently identified prostaglandin (PG) signaling as a cascade linking high wall shear stress with inflammation in IA walls[29]. Because inhibition of COX-2, enzyme for PGE_2 synthesis, or deficiency of one of PGE receptor subtype, EP2, significantly suppress IA formation in animal models through the inhibition of inflammation such as NF-κB activation in IA lesions[29], COX-2-PGE_2-EP2 signaling cascade plays the critical role for IA formation via regulating inflammation in intracranial arterial walls. Here, because COX-2 is shown to be induced under shear stress in endothelial cells[30], COX-2-PGE_2-EP2 signaling cascade is presumed to be induced under shear stress in intracranial arteries. Indeed, in primary endothelial cells from human carotid artery, laminar shear stress induces COX-2 and also EP2 expression at the force-dependent manner[29]. Consistent with these findings, COX-2 and EP2 induction is found in endothelial cells of IA walls from the early stage of IA formation confirming that COX-2-PGE_2-EP2 signaling cascade functions in these cells also in vivo probably under the shear stress[29]. Further, EP2 stimulation activates NF-κB in these cells *in vitro*, suggesting the regulation of NF-κB activation in endothelial cells by PGE_2-EP2 signaling[29]. Importantly, a deficit in PGE_2-EP2 signaling abolishes NF-κB activation in IA walls and vice versa[29]. Considered with the findings that NF-κB transcriptionally induces COX-2 expression, we propose that a positive feedback loop through COX-2-PGE_2-EP2-NF-κB signaling is formed under high wall shear stress in endothelial cells at the early stage of IA formation and contributes to the amplification and chronicity of inflammation associated with IA (Fig.1).

Inflammation in IA walls is not restricted only in endothelial cells of arterial walls but spreads into whole arterial walls at the late stage of IA formation[17, 18]. Recent experimental findings provide the evidence regarding the critical role of macrophages in spreads of inflammation in IA walls[20, 22, 23]. Macrophage is a major inflammatory cell infiltrated in IA walls of both human[6, 7, 31] and animal model[20]. MCP-1 is a key chemoattractant for macrophages to infiltrate affected

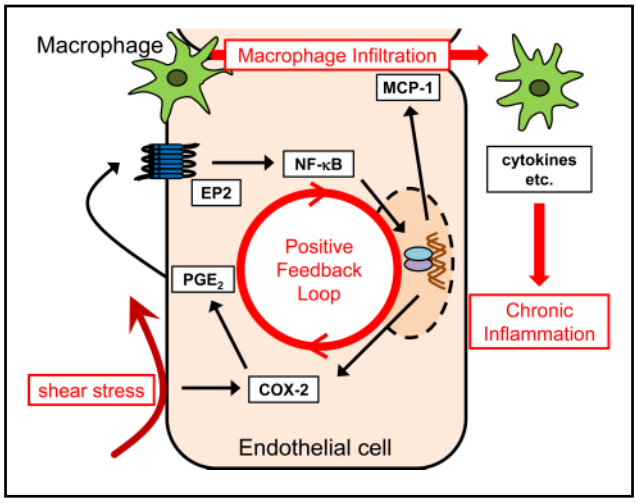

Fig.1 Schema of the proposed mechanisms regulating the long-lasting (chronic) inflammation contributing to IA formation

tissues[32]. Here, MCP-1-mediated macrophage infiltration in IA walls is critical for IA formation based on the findings that macrophage depletion by chlodronate liposome or blockage of macrophage infiltration into IA walls through MCP-1 deficiency or dominant negative form of MCP-1 suppress IA formation[22, 23]. Infiltrating macrophages, presumably via NF-κB-mediated MCP-1 induction in endothelial cells, exacerbate and spread the inflammation into whole arterial walls through secreting a wide range of pro-inflammatory molecules such as cytokines (IL-1β etc.)[19], chemoattractants for inflammatory cells (MCP-1 etc.)[23], tissue-destructive proteinases (MMP-9, cathepsins etc.)[20, 33], reactive oxygen[34] and so on (Fig.1). Furthermore, MCP-1 secreted from macrophages further recruit macrophages themselves and forms the autocrine loop resulting the long-lasting and exacerbated inflammation in IA walls[17].

Here, recent studies regarding the fluid hemodynamics have implied that low wall shear stress, not the high wall shear which triggers the IA formation, contributes to the enlargement of IA[35]. For example, in the region of enlargement, wall shear stress is significantly lower than that in surrounding aneurysm walls[35]. Considered with the findings that inhibition of inflammation in IA walls effectively suppresses not only the incidence but also the enlargement of IAs in animal models and that inflammatory responses including macrophage infiltration is augmented during IA formation[5, 18], the initiation and enlargement of IA may share common mechanisms in term of inflammation. However, there is no experi-

mental evidence regarding the molecular mechanisms linking low wall shear stress with the enlargement of IA. Because IA can cause devastating subarachnoid hemorrhage after rupture, the mechanism regulating the enlargement and, of course, the rupture of IA, should be carefully examined in near future to develop a novel therapeutic strategy for IA.

In summary, recent experimental findings mainly achieved using animal models of IAs provide the notion that IA is a chronic inflammatory disease of intracranial arteries. The mechanisms regulating the chronicity of inflammation are, first, the formation of positive feedback loop including COX-2-PGE_2-EP2-NF-κB signaling triggered by high wall shear stress at the early stage of IA formation, which amplifies the inflammation in endothelial cells, and, second, the infiltration of macrophages via NF-κB-mediated MCP-1 induction in endothelial cells in IA walls which spreads inflammation into whole IA walls.

Acknowledgements

We would like to express our sincere gratitude to all the researchers, collaborators, technical assistants and secretaries contributing to researches cited in the present manuscript. We also thank grants supporting our research works.

Source of funding

None

Conflict of interests

No conflict of interest to be disclosed.

References

1) Brisman JL, Song JK, Newell DW: Cerebral aneurysms. N Engl J Med. 2006; 355: 928-939.
2) van Gijn J, Kerr RS, Rinkel GJ: Subarachnoid haemorrhage. Lancet. 2007; 369: 306-318.
3) Wiebers DO, Whisnant JP, Huston J, 3rd, Meissner I, Brown RD, Jr., Piepgras DG, Forbes GS, Thielen K, Nichols D, O'Fallon WM, Peacock J, Jaeger L, Kassell NF, Kongable-Beckman GL, Torner JC: International Study of Unruptured Intracranial Aneurysms Investigators: Unruptured intracranial aneurysms: natural history, clinical outcome, and risks of surgical and endovascular treatment. Lancet. 2003; 362: 103-110.
4) Kataoka H, Aoki T: Molecular basis for the development of intracranial aneurysm. Expert Rev Neurother. 2010; 10: 173-187.
5) Aoki T, Nishimura M: Targeting chronic inflammation in cerebral aneurysms: focusing on NF-κB as a putative target of medical therapy. Expert Opin Ther Targets. 2010; 14: 265-273.
6) Chyatte D, Bruno G, Desai S, Todor DR: Inflammation and intracranial aneurysms. Neurosurgery. 1999; 45: 1137-1146.
7) Frosen J, Piippo A, Paetau A, Kangasniemi M, Niemela M, Hernesniemi J, Jääskeläinen J: Remodeling of saccular cerebral artery aneurysm wall is associated with rupture: histological analysis of 24 unruptured and 42 ruptured cases. Stroke. 2004; 35: 2287-2293.
8) Jayaraman T, Berenstein V, Li X, Mayer J, Silane M, Shin YS, Niimi Y, Kiliç T, Gunel M, Berenstein A: Tumor necrosis factor alpha is a key modulator of inflammation in cerebral aneurysms. Neurosurgery. 2005; 57: 558-564.
9) Krischek B, Kasuya H, Tajima A, Akagawa H, Sasaki T, Yoneyama T, Ujiie H, Kubo O, Bonin M, Takakura K, Hori T, Inoue I: Network-based gene expression analysis of intracranial aneurysm tissue reveals role of antigen presenting cells. Neuroscience. 2008; 154: 1398-1407.
10) Bruno G, Todor R, Lewis I, Chyatte D: Vascular extracellular matrix remodeling in cerebral aneurysms. J. Neurosurg. 1998; 89: 431-440.
11) Shojima M, Oshima M, Takagi K, Torii R, Hayakawa M, Katada K, Morita A, Kirino T: Magnitude and role of wall shear stress on cerebral aneurysm: computational fluid dynamic study of 20 middle cerebral artery aneurysms. Stroke. 2004; 35: 2500-2505.
12) Takeuchi S, Karino T: Flow patterns and distributions of fluid velocity and wall shear stress in the human internal carotid and middle cerebral arteries. World Neurosurg. 2010; 73: 174-185.
13) Jou LD, Lee DH, Morsi H, Mawad ME: Wall shear stress on ruptured and unruptured intracranial aneurysms at the internal carotid artery. AJNR Am J Neuroradiol. 2008; 29: 1761-1767.
14) Hashimoto N, Handa H, Hazama F: Experimentally induced cerebral aneurysms in rats. Surg Neurol. 1978; 10: 3-8.
15) Morimoto M, Miyamoto S, Mizoguchi A, Kume N, Kita T, Hashimoto N: Mouse model of cerebral aneurysm: experimental induction by renal hypertension and local hemodynamic changes. Stroke. 2002; 33: 1911-1915.
16) Aoki T, Nishimura M: The development and the use of

experimental animal models to study the underlying mechanisms of CA formation. J Biomed Biotechnol. 2011; 2011: 535921. doi: 10.1155/2011/535921.

17) Aoki T, Narumiya S: Prostaglandins and chronic inflammation. Trends Pharmacol Sciences. 2012; 33: 304-311.

18) Aoki T, Kataoka H, Shimamura M, Nakagami H, Wakayama K, Moriwaki T, Ishibashi R, Nozaki K, Morishita R, Hashimoto N: NF-κB is a key mediator of cerebral aneurysm formation. Circulation. 2007; 116: 2830-2840.

19) Moriwaki T, Takagi Y, Sadamasa N, Aoki T, Nozaki K, Hashimoto N: Impaired progression of cerebral aneurysms in interleukin-1b-deficient mice. Stroke. 2006; 37: 900-905.

20) Aoki T, Kataoka H, Morimoto M, Nozaki K, Hashimoto N: Macrophage-derived matrix metalloproteinase-2 and -9 promote the progression of cerebral aneurysms in rats. Stroke. 2007; 38: 162-169.

21) Sadamasa N, Nozaki K, Hashimoto N: Disruption of gene for inducible nitric oxide synthase reduces progression of cerebral aneurysms. Stroke. 2003; 34: 2980-2984.

22) Kanematsu Y, Kanematsu M, Kurihara C, Tada Y, Tsou TL, van Rooijen N, Lawton MT, Young WL, Liang EI, Nuki Y, Hashimoto T: Critical roles of macrophages in the formation of intracranial aneurysm. Stroke. 2011; 42: 173-178.

23) Aoki T, Kataoka H, Ishibashi R, Nozaki K, Egashira K, Hashimoto N: Impact of monocyte chemoattractant protein-1 deficiency on cerebral aneurysm formation. Stroke. 2009; 40: 942-951.

24) Aoki T, Kataoka H, Ishibashi R, Nozaki K, Hashimoto N: Simvastatin suppresses the progression of experimentally induced cerebral aneurysms in rats. Stroke. 2008; 39: 1276-1285.

25) Aoki T, Kataoka H, Ishibashi R, Nozaki K, Hashimoto N: Nifedipine inhibits the progression of an experimentally induced cerebral aneurysm in rats with associated down-regulation of NF-κB transcriptional activity. Curr Neurovasc Res. 2008; 5: 37-45.

26) Nakatani H, Hashimoto N, Kang Y, Yamazoe N, Kikuchi H, Yamaguchi S, Niimi H: Cerebral blood flow patterns at major vessel bifurcations and aneurysms in rats. Journal of neurosurgery. 1991; 74: 258-262.

27) Meng H, Wang Z, Hoi Y, Gao L, Metaxa E, Swartz DD, Kolega J: Complex hemodynamics at the apex of an arterial bifurcation induces vascular remodeling resembling cerebral aneurysm initiation. Stroke. 2007; 38: 1924-1931.

28) Wang Z, Kolega J, Hoi Y, Gao L, Swartz DD, Levy EI, Mocco J, Meng H: Molecular alterations associated with aneurysmal remodeling are localized in the high hemodynamic stress region of a created carotid bifurcation. Neurosurgery. 2009; 65: 169-177.

29) Aoki T, Nishimura M, Matsuoka T, Yamamoto K, Furuyashiki T, Kataoka H, Kitaoka S, Ishibashi R, Ishibazawa A, Miyamoto S, Morishita R, Ando J, Hashimoto N, Nozaki K, Narumiya S: PGE_2-EP2 signalling in endothelium is activated by haemodynamic stress and induces cerebral aneurysm through an amplifying loop via NF-κB. Br J Pharmacol. 2011; 163: 1237-1249.

30) Okahara K, Sun B, Kambayashi J: Upregulation of prostacyclin synthesis-related gene expression by shear stress in vascular endothelial cells. Arterioscler. Thromb Vasc Biol. 1998; 18: 1922-1926.

31) Tulamo R, Frosen J, Junnikkala S, Paetau A, Pitkäniemi J, Kangasniemi M, Niemelä M, Jääskeläinen J, Jokitalo E, Karatas A, Hernesniemi J, Meri S: Complement activation associates with saccular cerebral artery aneurysm wall degeneration and rupture. Neurosurgery. 2006; 59: 1069-1076.

32) Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, North R, Gerard C, Rollins BJ: Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. J Exp Med. 1998; 187: 601-608.

33) Aoki T, Kataoka H, Ishibashi R, Nozaki K, Hashimoto N: Cathepsin B, K, and S are expressed in cerebral aneurysms and promote the progression of cerebral aneurysms. Stroke. 2008; 39: 2603-2610.

34) Aoki T, Nishimura M, Kataoka H, Ishibashi R, Nozaki K, Hashimoto N: Reactive oxygen species modulate growth of cerebral aneurysms: a study using the free radical scavenger edaravone and $p47phox^{-/-}$ mice. Lab Invest. 2009; 89: 730-741.

35) Boussel L, Rayz V, McCulloch C, Martin A, Acevedo-Bolton G, Lawton M, Higashida R, Smith W, Young W, Saloner D: Aneurysm growth occurs at region of low wall shear stress: patient-specific correlation of hemodynamics and growth in a longitudinal study. Stroke. 2008; 39: 2997-3002.