Alternatively Activated Macrophages Play an Important Role in Vascular Remodeling and Hemorrhaging in Patients with Brain Arteriovenous Malformation

Yukihiko Nakamura, MD,*† Yasuo Sugita, MD,† Shinji Nakashima, MD,*† Yousuke Okada, MD,* Munetake Yoshitomi, MD,* Yoshizou Kimura, MD,† Hiroaki Miyoshi, MD,† Motohiro Morioka, MD,* and Koichi Ohshima, MD†

Background: Angiogenic and immunoactive lesions in brain arteriovenous malformation (BAVM) contribute to hemorrhagic events and the growth of BAVMs. However, the detailed mechanism is unclear. Our objective is to clarify the relationship between hemorrhagic events of BAVM and alternatively activated macrophages in the perinidal dilated capillary network (PDCN). *Methods:* We examined microsurgical specimens of BVMs (n = 29) and focused on the PDCN area. Ten autopsied brains without intracranial disease were the controls. We performed immunostaining of the inflammatory and endothelial cell markers, macrophage markers (CD163 and CD68), and vascular endothelial growth factor A (VEGF-A). We evaluated each cell's density and the vessel density in the PDCN and analyzed the relationship to hemorrhagic events of BAVM. *Results:* The PDCN was involved in all the resected arteriovenous malformations, and these vessels showed a high rate of CD105 expression (72.0 ± 10.64%), indicating newly proliferating vessels. Alternatively activated macrophages were found, with a high rate (85.6%) for all macrophages (controls, 56.6%). In the hemorrhagic cases, the cell density was significantly higher than that in the nonhemorrhagic cases and controls (hemorrhagic group, 290 ± 44 cells/mm^2; nonhemorrhagic group, 180 ± 59 cells/mm^2; and control, 19 ± 8 cells/mm^2). The cell density of alternatively activated macrophages showed a positive correlation with the vessel density of the PDCN. Double immunostaining showed that VEGF-A was secreted by alternatively activated macrophages. *Conclusion:* Our data suggest that alternatively activated macrophages may have some relationships with angiogenesis of PDCN and hemorrhagic event of BAVM. **Key Words:** Arteriovenous malformation—alternatively activated macrophage—perinidal dilated capillary network—vascular remodeling—VEGF.

From the *Department of Neurosurgery, Kurume University of School of Medicine, Japan; and †Department of Pathology, Kurume University of School of Medicine, Japan.

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Address correspondence to Motohiro Morioka, MD, Department of Neurosurgery, Kurume University of School of Medicine, 67, Asahi-machi, Kurume, Fukuoka, Japan. E-mail: mmorioka@med.kurume-u.ac.jp.

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Introduction

Brain arteriovenous malformation (BAVM) is a major cause of hemorrhagic stroke in young patients. Therefore, a key therapeutic strategy is the prevention of bleeding and rebleeding. Multimodal therapies such as gamma knife, endovascular embolization, surgical resection, and a combination of these therapies are available.[1] However, some patients are not able to receive these treatments because of untreatable lesions (e.g., a large size, eloquent area, and deep drainer).[2] In addition, even if BAVM appears to be resolved, it sometimes recurs or rebleeds.[3] Thus, it is essential to clarify the pathogenesis and mechanism of rupture in BAVM. Although a satisfactory sporadic BAVM animal model does not exist, some recent reports have described the pathogenesis of BAVM.

Recently, some investigators demonstrated that areas containing dilated vessels existed in the perinidal space (i.e., the perinidal dilated capillary network [PDCN]). In a 3-dimensional anatomical study, Sato et al[4] reported that the PDCN is connected to the nidus, feeding arteries, and draining veins via the arterioles and venules, as well as to the normal capillaries, arterioles, and venules. Tu et al[5] reported that the perinidal capillaries demonstrated an abnormal ultrastructure of the blood–brain barrier with no basement membranes or astrocytic foot processes. These vessels are related to intraoperative and postoperative hemorrhages.[4,5] Some studies have reported that angiogenic or cell proliferating factors and signals (vascular endothelial growth factor [VEGF], platelet-derived growth factor, notch, and nuclear factor-κB) were detected in human BAVM samples.[6-9] Furthermore, endothelial cells of arteriovenous malformation (AVM) showed more frequent apoptosis and proliferation than normal controls in a culture study.[10] Therefore, BAVM and perinidal dilated capillaries are not considered static congenital lesions; instead, they are dynamically growing (or changing) lesions of their own.[11]

Chen et al[12] described that an aberrant immune response was an important factor of vascular remodeling in the BAVM microenvironment. They also found that macrophages and neutrophils infiltrate more frequently than lymphocytes in BAVM tissue. Recent studies showed that the macrophages act as cellular chaperones for vascular anastomosis and remodeling of the extracellular matrix.[13,14] However, it is unclear whether these macrophages are associated with vascular remodeling in BAVM tissue.

Macrophage activation is clearly divided into 2 pathways: classical activation (changing to M1 phenotype) and alternative activation (changing to M2 phenotype).[15-18] Classical activation is induced by bacterial products (e.g., lipopolysaccharides) and interferon-γ. In contrast, alternative activation is induced by Th2-type cytokines such as interleukin (IL)-4, IL-10, IL-13, vitamin D3, the macrophage colony stimulation factor, or a glucocorticoid.[15-18] Alternatively activated macrophages/microglia are associated with a high degree of vascularization, which influences wound repair.[19,20] In some malignant tumors, alternatively activated macrophages are tumor-associated macrophages (TAMs), which are associated with poor prognosis in breast, colon, prostate, glioma, and cervical cancers. TAMs promote invasion, growth, angiogenesis, and metastasis of malignant tumors.[21-25]

Our study focused on alternatively activated macrophages (i.e., M2 macrophage or TAMs) in BAVM. We used the antibody (Ab) for CD163, a member of the scavenger receptor cysteine-rich superfamily restricted to monocyte/macrophage lineage, which is a useful marker for anti-inflammatory or alternatively activated macrophages.[26] We aimed to investigate the origin of the dilated capillary network, analyze the macrophage phenotype and function, and clarify the relationship between macrophages and dilated capillary vessel formation in surgically resected BAVM samples.

Methods

Patients

We surgically removed 28 BAVMs at the Kurume University Hospital between 1996 and 2010. The clinical data are summarized in Table 1; 19 patients were hemorrhagic cases and 9 were nonhemorrhagic cases. Ten autopsied brains without intracranial disease were the controls. The patients' mean age was 43.1 ± 12.4 years, and 11 were female. In the nonhemorrhagic cases, 3 patients had AVM at seizure onset, while the other AVMs were discovered incidentally. The nidus size ranged from 8 to 55 mm (mean, 25.3 mm). Four patients' AVMs were Spetzler–Martin grade I, 15 were grade II, and 10 were grade III. Of all 19 hemorrhagic cases, 15 (78.9%) cases underwent acute phase removal surgery (within 48 hours after the hemorrhagic event).

Abs

The following Abs were used in the present study: anti-CD3 Ab as a T-lymphocyte marker (1:100; Novocastra, Leica Biosystems, Newcastle, UK), anti-CD20 Ab as a B-lymphocyte marker (1:1000; Dako Corporation, Carpinteria, CA), antimyeloperoxidase Ab as a neutrophil marker (1:4000, Dako Corporation), rabbit and mouse anti-CD68 Abs as a macrophage marker (1:200; Dako, Grostrup, Denmark), anti-CD163 Ab (1:200, Novocastra) for recognizing macrophage-restricted membrane protein, anti-CD34 as a vascular endothelial cell marker (1:200; Immunotech, Marseille, France), antihuman CD105 Ab (antiendoglin, 1:200; Dako Corporation), and anti-VEGF (A20, sc-120) and antivascular endothelial growth factor A (VEGF-A) Abs (1:200; Santa Cruz Biotechnology, Santa Cruz, CA). In the present study, alternatively activated macrophages were recognized as