Supplemental Material

Critical roles of macrophages in the formation of intracranial aneurysm

Authors

Yasuhisa Kanematsu, MD PhD, [1,4,5], Miyuki Kanematsu, MD[1,4,6], Chie Kurihara, BS[1,4], Yoshiteru Tada, MD, PhD[1,4,5], Tsung-Ling Tsou, BS[1,4], Nico van Rooijen, MD, Michael T. Lawton, MD[7], William L. Young, MD[1,2,3,4], Elena I. Liang, BS[1,4], Yoshitsugu Nuki, MD PhD,[1,4], Tomoki Hashimoto, MD[1,4]

Department of Anesthesia and Perioperative Care[1], Neurology[2] and Neurosurgery[3], Center for Cerebrovascular Research[4], University of California, San Francisco, California, U.S.A.

Department of Neurosurgery[5], and Department of Thoracic, Endocrine Surgery and Oncology[6], Institute of Health Biosciences, University of Tokushima, Tokushima, Japan

Department of Molecular Cell Biology[7], Vrije Universiteit, Amsterdam, The Netherlands

Correspondence to: Tomoki Hashimoto, MD, Department of Anesthesia and Perioperative Care, University of California, San Francisco, 1001 Potrero Avenue, No. 3C-38, San Francisco, CA 94110, USA. Phone: (415) 206-8958. Fax: (415) 206-8170; Email: hashimot@anesthesia.ucsf.edu

Immunohistochemical analysis

Immunohistochemical staining was performed using rat monoclonal anti mouse CD45 for pan-leukocytes and rat monoclonal anti mouse CD68 for macrophages as previously described.[1] Representative aneurysms from the wild-type mice that received elastase and angiotensin-II treatment and control middle cerebral artery (immediately distal of the bifurcation from the internal carotid artery) from the wild-type mice that received a stereotaxic injection of PBS and a continuous infusion of PBS through osmotic pump were used to identify inflammatory cell types that infiltrated into aneurysms.

For quantification of macrophages infiltration into cerebral arteries, an additional five mice in each group (clodronate liposome, PBS liposome, MCP-1 knockout mice, MMP-12 knockout mice, and wild-type mice) were sacrificed one week after aneurysm induction. One cross-sectional slice of the middle cerebral artery, immediately distal of the bifurcation from the internal carotid artery, from each mouse was used. Two blinded observers independently performed quantitative analysis as previously described.[1] Macrophages were counted under high magnification (400x) in a randomly selected area of each quadrant of the cross-section of the middle cerebral artery. The arterial area per field was measured by using ImageJ software (National Institutes of Health). The number of macrophages per area of 0.01 square millimeters was calculated using the following formula: number of positive cells per field / arterial area per field. Results from the two observers were averaged.

References

1. Kanematsu Y, Kanematsu M, Kurihara C, Tsou TL, Nuki Y, Liang EI, Makino H, Hashimoto T. Pharmacologically induced thoracic and abdominal aortic aneurysms in mice. *Hypertension*. 2010;55:1267-1274