The Phenotype of Infiltrating Macrophages Influences Arteriosclerotic Plaque Vulnerability in the Carotid Artery

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Background: Proinflammatory (M1) macrophages and anti-inflammatory (M2) macrophages have been identified in atherosclerotic plaques. While these macrophages have been speculated to be related to plaque vulnerability, there are limited studies investigating this relationship. Therefore, we examined the association between macrophage phenotype (M1 versus M2) and plaque vulnerability and clinical events. *Methods:* Patients undergoing carotid endarterectomy received an ultrasound of the carotid artery before surgery. Plaques were processed for analysis by immunohistochemistry, Western blotting, and real-time polymerase chain reaction studies. Medical history and clinical data were obtained from medical records. *Results:* Patients were divided into 2 groups: those suffering from acute ischemic attack (symptomatic, n = 31) and those that did not present with symptoms (asymptomatic, n = 34). Ultrasound analysis revealed that plaque vulnerability was greater in the symptomatic group ($P = .033$; Chi-square test). Immunohistochemistry revealed that plaques from the symptomatic group had a greater concentration of M1 macrophages (CD68-, CD11c-positive) while plaques from the asymptomatic group had more M2 macrophages (CD163-positive). This observation was confirmed by Western blotting. Characterization by real-time polymerase chain reaction studies revealed that plaques from the symptomatic group had increased expression of the M1 markers CD68 and CD11c, as well as monocyte chemoattractive protein-1, interleukin-6, and matrix metalloproteinase-9. In addition, more M1 macrophages expressed in unstable plaques were defined by ultrasound analysis, while more M2 macrophages were expressed in stable plaques. *Conclusions:* Our data show that M1 macrophage content of atherosclerotic plaques is associated with clinical incidence of ischemic stroke and increased inflammation or fibrinolysis. We also show the benefits of using ultrasound to evaluate vulnerability in the plaques. **Key Words:** Atherosclerosis—carotid artery disease—inflammation—macrophage—stroke.

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Atherosclerotic disease in the carotid artery is one important cause of ischemic stroke. Ischemic stroke is estimated to be responsible for >10% of all deaths and is the second cause of mortality worldwide.[1] Atherosclerosis is a progressive and chronic inflammatory disease in which lipids, immune cells, vascular smooth muscle cells, and extracellular matrix accumulate in the subendothelial space to form the growing atherosclerotic lesion.[2] The inflammatory reaction taking place in plaques relies on highly complex processes that are still not completely understood. However, it is known that recruited leukocytes are key facilitators of these events. In mononuclear phagocytes, the so-called M1 and M2 activation pathways are thought to represent the embodiment of the control switches in innate immune response, creating a balance between a proinflammatory environment and an anti-inflammatory environment.[3] The M1 pathway is characterized by the synthesis of proinflammatory cytokines that are potentially harmful in the context of atheroma, while anti-inflammatory/reparative M2 macrophages are derived from monocytes activated along the alternative pathway.[4,5]

It is well known that the risk of cardiovascular events is related to the composition and stability of the plaque rather than to the degree of arterial stenosis, and recent studies suggest that inflammation is a critical determinant of plaque stability. Vulnerable plaque imaging techniques currently allow the analysis of plaque morphology and characteristics.[6-8]

In this study, we aim to investigate the relationship between macrophage polarity (M1 versus M2) and the vulnerability of human atherosclerotic plaques. Patients undergoing carotid endarterectomy (CEA) were divided into 2 groups: those suffering from cerebral infarction (symptomatic group) and those that did not present with symptoms (asymptomatic group). Plaques obtained from both populations were studied to determine the nature of the macrophages associated with the plaque and their association with atherosclerotic factors.

Methods

Patients

Between November 2008 and April 2011, 65 patients underwent CEA at Sapporo Asabu Neurosurgical Hospital, Nakamura Memorial Hospital, Sapporo City General Hospital, or Hokkaido University Hospital. Written informed consent from each patient was obtained before enrollment in the study, which was performed according to Good Clinical Practice and Helsinki Declaration principles. The ethics committees of each hospital and Hokkaido University approved the studies.

Inclusion Criteria

Patients who had high-grade (>70%) stenosis and were asymptomatic or intermediate stenosis (>50%) for symptomatic patients in the internal carotid artery according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[9] and Japanese Guidelines for the Management of Stroke criteria[1] confirmed by carotid B-mode ultrasound were collected. The clinical indication for CEA was met after examination by the neurologist based on the guidelines.

Patient Characteristics, History, and Medication

Medical history was recorded from all patients, and the presence of vascular risk factors, features of metabolic syndrome, and medications were noted. Clinical history was assessed for diabetes mellitus, smoking, hypertension, dyslipidemia, previous acute cerebrovascular or myocardial infarction, and peripheral vascular disease. Hypertension was diagnosed according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) and the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009) criteria (blood pressure ≥140/90 mm Hg or ≥130/80 mm Hg [with diabetes mellitus] or current antihypertensive treatment).[10] Diabetes was diagnosed in patients with dietary treatment, those taking antidiabetic medications, or those with current fasting plasma glucose levels >7.0 mmol/L. Diagnosis of dyslipidemia was made according to the Japan Atherosclerosis Society Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2007 when ≥1 of the following lipid abnormalities was present: low-density lipoprotein cholesterol (LDL-C) ≥3.6 mmol/L (LDL-C was determined using the Friedewald equation), high-density lipoprotein cholesterol <1.0 mmol/L, triglycerides ≥1.7 mmol/L, or when a patient was taking a lipid-lowering drug. Fasting blood samples were taken before CEA for analysis (Table 1).

Definition of Symptomatic Carotid Disease

Patients were categorized by their physician as either symptomatic or asymptomatic based on an evaluation of their history and a clinical examination. Patients who had acute onset focal neurologic symptoms were considered symptomatic, and the median number of days between the acute clinical events and the CEA was 21.5 days (range 8-40 days) in the symptomatic group. Patients who experienced acute clinical events more than 6 months earlier were considered asymptomatic.

Carotid B-mode Ultrasound

The carotid arteries were carefully examined before surgery with commercially available equipment (GE Healthcare LOGIQ9 with 9-MHz linear array transducer; Waukesha, WI). Echo imaging and the evaluation of the degree of stenosis was performed by a well-trained operator who was unaware of the clinical profile of the