Localized Increase of Chemokines in the Lumen of Human Cerebral Aneurysms

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Background and Purpose—Inflammation may play an important role in the formation and rupture of cerebral aneurysms. Chemokines act as chemoattractants for leukocytes directing them toward sites of tissue inflammation. The purpose of this study was to determine whether chemokines and chemoattractant cytokines were increased in the lumen of human cerebral aneurysms.

Methods—The concentrations of chemokines and other inflammatory molecules in blood samples drawn from the lumen of human cerebral aneurysms of 16 consecutive patients (harboring 18 aneurysms) were compared with blood samples from the femoral arteries of the same patients. Three aneurysms had ruptured.

Results—The mean plasma concentration of regulated on activation, normal T cell expressed and secreted (RANTES), monokine-induced-by-γ-interferon (MIG), interferon-γ-induced protein-10 (IP-10), eotaxin, interleukin (IL) 8, and IL17 was significantly higher in samples taken from cerebral aneurysms compared with femoral arteries. In contrast, plasma concentrations of all remaining inflammatory molecules (except IL6) that were tested did not differ between cerebral aneurysms and femoral arteries. For unruptured aneurysms, there was a significantly higher mean plasma concentration of monocyte chemoattractant protein-1 as well as RANTES, MIG, IP-10, eotaxin, IL8, and IL17 in samples obtained from cerebral aneurysms.

Conclusions—High plasma concentrations of chemokines (monocyte chemoattractant protein-1, RANTES, MIG, IP-10, and eotaxin) and chemoattractant cytokines (IL8 and IL17) were found in the lumen of human cerebral aneurysms. These findings suggest that there may be an active recruitment of inflammatory cells into the aneurysm wall that may be exploited therapeutically. (Stroke. 2013;44:2594-2597.)

Key Words: aneurysm ■ chemokines ■ cytokines ■ inflammation

Inflammation may play a key role in the formation and rupture of cerebral aneurysms. Several constituents of the inflammatory response seem to be involved, including leukocytes, vascular smooth muscle cells, cytokines, growth factors, reactive oxygen species, and matrix metalloproteinases. Chemokines are a family of low molecular weight cytokines that act as chemoattractants to guide the migration of leukocytes toward the site of inflammation. Although several studies have shown that inflammatory cells are recruited and proliferate in the walls of cerebral aneurysms, there have been no reports about the role of chemokines in human cerebral aneurysms. In this study, we sought to determine whether there is an increased chemokine and chemoattractant cytokine expression in the lumen of human cerebral aneurysms.

Methods

Participants
The study protocol was approved by the University of Iowa Institutional Review Board. All patients presented to the Department of Neurosurgery at the University of Iowa Hospitals and Clinics between November and December 2012. Consecutive patients harboring saccular intracranial aneurysms (ruptured or unruptured) who were candidates for coil embolization were prospectively enrolled in the study. Patients taking corticosteroids or immunosuppressant therapy were excluded. A total of 16 patients harboring 18 aneurysms were enrolled.

Cerebral Angiography and Blood Sampling
In each patient, arterial access was obtained through femoral puncture by use of the Seldinger technique, and a 7-French arterial sheath was inserted and secured with a stitch. A blood sample (5 ml) was subsequently drawn from the femoral artery. The guiding catheter was navigated into each studied vessel and the aneurysm was identified. A microcatheter was subsequently advanced over a microguidewire and placed in the aneurysm lumen. A blood sample (5 ml) was taken from the aneurysm lumen before coil deployment, centrifuged, and the plasma was stored at −80°C until analysis. All samples, from both the femoral artery and the aneurysm lumen, were immediately frozen after collection. As such, there was no time difference between specimen collection and freezing for the 2 sites.

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Biochemical Measurements

The plasma concentrations of several chemokines, cytokines, and growth factors in aneurysm and femoral samples were quantified with Luminex-based immunoassay. The concentrations of the following molecules were determined: regulated on activation, normal T cell expressed and secreted (RANTES), monokine-induced-by-γ-interferon (MIG), interferon-γ-induced protein-10 (IP-10), eotaxin, interleukin (IL) 1-β, IL1 receptor antagonist, IL2, IL2 receptor, IL4, IL5, IL6, IL10, IL12, IL13, IL15, IL17, vascular endothelial growth factor, granulocyte colony-stimulating factor, epidermal growth factor, basic fibroblast growth factor, granulocyte monocyte colony-stimulating factor, tumor necrosis factor-α, interferon-α, interferon-γ, macrophage inflammatory protein-1-α, macrophage inflammatory protein-1-β, and monocyte chemoattractant protein-1 (MCP-1).

Statistical Analysis

Data are presented as mean and SD for continuous variables, and as frequency for categorical variables. Analysis of plasma levels of chemokines and inflammatory molecules was carried out using paired t test. P values of ≤0.05 were considered statistically significant.

Results

Of the 16 patients, 13 were women and 3 were men with a mean age of 55±13 years. Aneurysm size was 10±9 mm on average. Three of the 18 aneurysms (16%) had ruptured. Only 1 patient (with a giant aneurysm) had evidence of a mural thrombus. Table 1 summarizes patient demographics and aneurysm characteristics.

The mean plasma concentration of RANTES, MIG, IP-10, eotaxin, IL8, and IL17 was significantly higher in samples obtained from cerebral aneurysms compared with IP-10 (28.6±12.9 versus 13.7±1.9; P=0.03), IP-10 (25.2±11.9 versus 15.7±7.6; P=0.002), eotaxin (45.6±24.4 versus 38.6±15.4; P=0.03), IL8 (23.5±3.5 versus 21.5±3.2; P=0.05), and IL17 (42.4±2.7 versus 40.3±3.05; P=0.005), was found to be significantly higher in samples obtained from cerebral aneurysms compared with the femoral artery.

Discussion

Aneurysm formation is thought to occur after hemodynamic insult, which elicits a series of proinflammatory changes in endothelial cells.1,6 This is followed by the infiltration, activation, and proliferation of inflammatory cells, including monocytes, lymphocytes, neutrophils, and mast cells. Tada et al7 noted early endothelial damage with interendothelial gaps at the site of cerebral aneurysm and demonstrated that the disruption of endothelial tight junctions was associated with the migration of leukocytes into aneurysm walls. The mounting inflammatory response leads to the release of matrix metalloproteinases and apoptosis of cellular constituents of the vessel wall, which ultimately results in aneurysm wall weakening and rupture.1

Macrophages and neutrophils may play a key role in aneurysm formation and rupture through flow-induced vascular remodeling (release of metalloproteinases).1,8 Macrophage infiltration correlates with the risk of cerebral aneurysm rupture in humans and macrophage depletion halts aneurysm formation in mice.2,9 MCP-1, Ets-1, and nuclear factor kappa B were found to be pivotal in recruiting macrophages into the wall of cerebral aneurysms. T cells are also frequently present in the wall of cerebral aneurysms, and their presence is associated with aneurysm rupture.10,11 Mast cells may play a role in aneurysm formation and rupture. Ishibashi et al12 found an increased number of mast cells during the formation of cerebral aneurysms and were able to halt aneurysm progression with a mast cell degranulation inhibitor. We have previously demonstrated that upregulation of mast cells along with macrophage M1/M2 imbalance is associated with the rupture human cerebral aneurysms.13

The cytokines that have been found to be prominently involved in the pathogenesis of cerebral aneurysms are IL1β, IL6, and tumor necrosis factor-α.1 The only chemokine shown thus far to have a role in aneurysm formation and progression is MCP-1, a chemoattractant of macrophages. Aoki et al14 found that MCP-1 expression was upregulated in aneurysmal walls at the early stage of cerebral aneurysm formation in rats and that blockade of MCP-1 resulted in a significant decrease in macrophages infiltration, inflammatory changes, and aneurysm progression. On the basis of these data, the authors concluded that MCP-1 plays a crucial role in cerebral aneurysm formation as a major chemoattractant for monocyte/macrophage. In the present study, MCP-1 level was higher in the lumen of unruptured cerebral aneurysms which corroborates the findings of Aoki et al.14 We also found that the plasma levels of 6
chemokines and chemoattractant cytokines, namely RANTES, MIG, IP-10, eotaxin, IL8, and IL17, were particularly high in the lumen of human intracranial aneurysms as compared with femoral arteries. These findings provide the first evidence that these chemokines and chemoattractant cytokines may play a role in the pathogenesis of human cerebral aneurysms through active recruitment of inflammatory cells. The fact that the concentrations of other inflammatory molecules were comparable between the 2 arterial sites lends further credence to our findings. We postulate that these chemokines and chemoattractant cytokines are secreted by endothelial cells and leukocytes (including macrophages) in the wall of the aneurysms.

RANTES also known as chemokine (CC motif) ligand 5 is chemotactic for T cells, eosinophils, and basophils, whereas MIG also known as chemokine (CXC motif) ligand 9 serves as an interferon-γ–induced cell chemoattractant. IP-10 also known as C-X-C motif chemokine 10 is another chemokine induced by interferon-γ and has several roles, most importantly the chemoattraction of monocytes/macrophages and T cells. Eotaxin is a specific chemoattractant for eosinophil cells and IL8 induces chemotaxis in neutrophils. Finally, IL17 is a cytokine whose main role is to increase chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation. These functions are particularly relevant with regard to cerebral aneurysms because, as discussed above, the infiltration of T cells, macrophages, neutrophils, and other inflammatory cells is a hallmark of formation and rupture of cerebral aneurysms.

Limitations
The relatively small number of patients enrolled is a limitation of this study. Despite this limitation, sufficient data were obtained to demonstrate the overexpression of chemokines in the lumen of human cerebral aneurysms. For subgroup analysis (unruptured aneurysms), the difference in chemokine concentrations was more modest between the 2 sites. However, it remained statistically significant, despite the small number of patients included. It is possible that some of the changes in chemokines expression in cerebral aneurysms may be compensatory in nature and act to prevent further deterioration. For example, MIG and IP-10 are proposed to have angiostatic function and as such could theoretically assist in inhibiting aneurysm growth. A relationship, however, between angiogenesis and cerebral aneurysms has yet to be demonstrated and most proposed mechanisms for aneurysm formation and rupture do not include angiogenesis as a critical pathway. Although blood stagnation, platelet aggregation, and thrombus formation are common in the lumen of giant cerebral aneurysms, the inflammatory profile shown in the present study differs from that of platelet-derived factors alone. For example, IL1-β, epidermal growth factor, and basic fibroblast growth factor are 3 important molecules released by platelets that were not found to be elevated in aneurysm lumens in the present study. Moreover, only 1 aneurysm was giant and had evidence of mural thrombus.

We compared the concentration of inflammatory molecules between the systemic circulation (femoral artery) and the local aneurysm microenvironment. One could argue that the observed difference in cytokine composition may be, in part, because of the origin of blood—peripheral vessel versus intracerebral—rather than the aneurysm itself. This is, however, unlikely given the selective profile of these changes which indicates a specific pathogenic process rather than a difference simply because of the origin of blood.

Conclusions
In this study, we found high concentrations of RANTES, MIG, IP-10, eotaxin, IL8, and IL17 in the lumen of human cerebral aneurysms (in addition to MCP-1 for unruptured aneurysms). The high concentration of these chemokines suggests active recruitment of inflammatory cells to the aneurysm wall and possible therapeutic target of medical treatment for prevention of cerebral aneurysm formation and rupture.
Disclosures
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References
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