Anti-α-Fodrin Autoantibodies in Moyamoya Disease

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Background and Purpose—Moyamoya disease (MMD) is a rare entity that results in progressive occlusion of the arteries of the circle of Willis, but the pathogenesis of MMD is unknown.

Methods—MMD sera (n = 32) were tested for anti-endothelial cell antibodies by enzyme-linked immunoassays and flow cytometric analysis. Apoptosis was induced in human umbilical vein endothelial cells by tumor necrosis factor-α.

Results—We found that a high proportion of MMD sera had anti-endothelial cell antibodies with apoptotic stimuli. Prominent reactivities of MMD sera (72%) with recombinant human α-fodrin were observed.

Conclusions—Our study demonstrates that MMD sera contain a high incidence of anti-α-fodrin autoantibodies, providing new insight into the mechanisms of occlusion of MMD arteries. (Stroke. 2003;34:e244-e246.)

Key Words: α-fodrin ■ autoantibodies ■ moyamoya disease

Moyamoya disease (MMD) is a chronic cerebrovascular occlusive disease first reported by Japanese surgeons.1 The disease is characterized by stenosis or occlusion of the terminal portions of the bilateral internal carotid arteries and an abnormal vascular network referred to as moyamoya vessels.2 Although the cause of MMD remains undetermined, evidence supports an infectious origin, suggesting a role for bacterial and viral infections.3,4 It was also reported that MMD itself has been associated with Sjögren’s syndrome5 and anti-phospholipid autoantibodies.6

It was demonstrated that a defined set of cytoskeletal and nuclear proteins, including α-fodrin and poly(ADP-ribose) polymerase (PARP), were selectively cleaved during apoptosis induced by various stimuli.7 These findings suggest that different proteases act in apoptosis and that, although cell death processes result in selective cleavage of almost identical cellular proteins, they can be distinguished on the basis of their cleavage products. The purpose of the present study was to seek evidence for autoantibodies against apoptosis-related proteins in patients with MMD.

Subjects and Methods

Study Patients

This study included 32 MMD patients confirmed by cerebral angiography, CT scans, or MRI scans (the Table). Comparative studies were performed with systemic sclerosis (SSc) patients (n = 16).

Cell Culture and Induction of Apoptosis

Human umbilical vein endothelial cells (HUVECs) were purchased from Bio Whittaker. Apoptosis was induced in HUVEC by tumor necrosis factor (TNF)-α (100 ng/mL, R&D Systems) and determined by an EPICS flow cytometer (Coulter) with the Mitochondrial Apoptosis Detection Kit (Biovision).

Enzyme-Linked Immunosorbent Assay for Anti-Endothelial Cell Antibodies

Enzyme-linked immunosorbent assay (ELISA) for anti-endothelial cell antibodies (AECAs) was performed as described.8 Optical density was measured at 495 nm in a Titertek Uniskan (Flow Labs). Absorbance values greater than the mean ±3 SD in normal controls were considered positive.

Flow Cytometric Analysis for AECA With Apoptosis

Apoptotic HUVECs were incubated with sera diluted to 1:20 in bovine serum albumin/phosphate-buffered saline. Cells were analyzed on an EPICS flow cytometer (Coulter). Samples were recorded as positive if the binding index was greater than the mean ±3 SD of the normal group.

Western Blot Analysis

Western blot analysis with mouse mAb to α-fodrin (AFFINITI, Mamhead), PARP (Transduction Laboratories), gelsolin (DAKO), and active caspase 3 (Transduction Laboratories) was performed and visualized with ECL. Western blotted reagent (Amersham Corp). Recombinant caspase 3 was purchased from Biovision, and recombinant α-fodrin was constructed by inserting cDNA into the EcoRI site of pGEX-4Ts.9

Results

ELISA for AECAs

IgG AECAs were detected in 2 of the 32 MMD patients, not in 32 control subjects (Figure 1A). IgG AECAs were present in 8 of the 16 patients with SSc (50%) (P<0.0001).
Flow Cytometric Analysis for AECAs With Apoptosis

Cytoplasmic staining was observed in a high proportion of SSc (P<0.0001) and MMD (P<0.001) patients positive for IgG AECAs with apoptosis (Figure 1B). Proteolysis of α-fodrin to 150- and 120-kDa breakdown products was detected in TNF-α-stimulated HUVECs (Figure 1C).

Anti-Human 120-kDa α-Fodrin Abs in MMD Sera
A high proportion of sera from MMD patients (72%) reacted with each recombinant α-fodrin compared with control subjects (13%) (Table). Serum reactivities with breakdown products of PARP were not observed. Strong reactivity of MMD sera with each recombinant human α-fodrin was observed, but not in sera from SSc patients (Figure 2A). A large proportion of MMD sera reacts with C-termini of recombinant α-fodrin protein (JS-1, 2.7A, 3’DA, 59%). Cleavage products (150 and 120 kDa) of rat brain α-fodrin were detected when treated with recombinant caspase 3, and MMD sera reacted with either 150- or 120-kDa but not with 240-kDa mature-form (Figure 2B). Moreover, TNF-α-stimulated HUVECs were positive for active caspase 3 (Figure 2C), and the cleavage products of α-fodrin were entirely blocked by preincubation with caspase inhibitors (z-VAD-fmk, DEVD-CHO) (Figure 2D).

Discussion
A number of studies have suggested that endothelial cell injury results in an altered distribution of surface Ag and promotes active binding of immune complexes to these cells. AECAs are reported to be closely correlated with the vasculitis in Kawasaki disease and Takayasu arteritis, suggesting that AECAs could contribute to the pathogenesis of vascular injury.

The new information obtained here is the presence of AECAs with apoptotic stimuli in MMD patients. ELISAs performed with conventional AECAs in the MMD patients were almost negative, indicating that no antibodies directed against endothelial cells bind primarily to membrane-bound molecules. However, sera from MMD patients contain autoantibodies against cleaved product of 150- or 120-kDa α-fodrin derived from apoptotic HUVECs. In vitro study demonstrated that MMD sera react with either 150- or 120-kDa but not with 240-kDa mature-form α-fodrin, which was cleaved by the recombinant caspase 3. This is the first report that autoantibodies cleave products of α-fodrin derived from apoptotic endothelial cells in MMD patients. It was demonstrated that the fodrin α subunit is cleaved in association with apoptosis and that the 120-kDa fragment is a breakdown product of the mature form of 240-kDa fodrin α.

### Frequency of α-Fodrin–Reactive Sera From MMD Patients and Age-Matched Healthy Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients, n</th>
<th>Mean Age (Range), y</th>
<th>Sex Ratio, F/M</th>
<th>Positive Sera With α-Fodrin, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>JS-1</td>
</tr>
<tr>
<td>MMD</td>
<td>32</td>
<td>33±17</td>
<td>27/5</td>
<td>9/32*</td>
</tr>
<tr>
<td>(12–66)</td>
<td>(5.4/1)</td>
<td></td>
<td></td>
<td>(28)</td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>30±11</td>
<td>27/5</td>
<td>1/32</td>
</tr>
<tr>
<td>(13–53)</td>
<td>(5.4/1)</td>
<td></td>
<td></td>
<td>(3)</td>
</tr>
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</table>

Statistically significant at *P<0.01, †P<0.001, and ‡P<0.0001 vs healthy control subjects (Mann-Whitney U test).

### Figure 1.
A, IgG AECAs were significantly higher in sera of SSC patients than control subjects (P<0.0001, Mann-Whitney U test). B, A high proportion of SSC and MMD patients were positive for IgG AECAs using apoptotic HUVECs compared with control subjects (P<0.0001 and P<0.001, respectively, Mann-Whitney U test). C, Proteolysis of α-fodrin to 150- and 120-kDa breakdown products was detected in TNF-α (100 ng/mL)–stimulated HUVECs. Treatment with TNF-α (100 ng/mL) affected breakdown of PARP (85 kDa) and gelsolin (cleavage product not detected).
subunit. A higher proportion of MMD sera reacts with C-termini of α-fodrin containing caspase 3 cleavage sites. Indeed, we detected active caspase 3 in apoptotic HUVECs, and cleavage products of α-fodrin were blocked by caspase inhibitors. The activation and injury of endothelial cells induced by TNF-α and other proinflammatory cytokines may underlie the local effects of these mediators in vivo. These data suggest that anti-α-fodrin autoantibody could contribute in part to the pathogenesis of MMD and may provide new insight into the mechanisms of occlusion of the arteries.

References
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