Immune complexes and complement activation following rupture of intracranial saccular aneurysms

JOHN R. ØSTERGAARD, M.D., BENT Ø. KRISTENSEN, M.D., SVEN-ERIK SVEHAG, D.V.M., PH.D., BØRGE TEISNER, M.D., AND TOMISLAV MILLETIC, M.D.

Departments of Neurosurgery, Cardiology, and Neuroradiology, Aarhus Kommunehospital, Aarhus, and Institute of Medical Microbiology, Odense University, Odense, Denmark

Circulating immune complexes (CIC) and complement activation (plasma C3d levels) were monitored during a 2-week period in patients with ruptured cerebral aneurysms and also in patients with cerebral hematoma unrelated to saccular aneurysms. Thirteen of 18 aneurysm patients were found to have CIC on admission as compared to three of 21 healthy blood donors (p < 0.001). The presence of CIC in aneurysm patients was associated with a poor prognosis. Eight of nine patients who developed angiographic vasospasm had CIC on admission compared with one of four without vasospasm. Patients with vasospasm showed a twofold increase in plasma C3d levels at the time when the spasm occurred, whereas no significant changes in the C3d concentration could be demonstrated in aneurysm patients without spasm or in patients with hematoma unrelated to aneurysm rupture. These findings suggest that immunological processes involving complement-activating immune complexes are involved in the pathogenesis of cerebral vasospasm following rupture of saccular aneurysms.

Key Words • aneurysm • vasospasm • complement activation • circulating immune complexes • subarachnoid hemorrhage

Cerebral vasospasm following subarachnoid hemorrhage (SAH) due to a ruptured aneurysm is an important cause of cerebral ischemia, and is the leading cause of death and disability after aneurysm rupture.5,13,14 The pathogenesis of this condition is poorly understood, and previous studies have failed to yield conclusive evidence as to the causative agent(s) or the nature of the cerebral artery narrowing.14,19

An increased incidence of circulating immune complexes (CIC)27 and deposition of immunoglobulin (Ig)G and complement C3 in the cerebral arterial walls15 of patients with chronic vasospasm have been reported. This suggests that immunological reactions are involved in cerebral vasospasm. Circulating immune complexes are detected in a number of diseases, and their effects are primarily mediated by deposition in tissues and complement activation which causes an inflammatory cellular reaction, particularly in small vessels, renal glomeruli, and joints. A similar reaction has also been observed in and around cerebral vessels following aneurysm rupture.12,16,29

In the present study, CIC as well as complement activation were monitored in a series of patients with ruptured saccular aneurysms. The findings were correlated to the outcome of the patients and to the development of angiographic vasospasm. Another group of patients with intracerebral hematoma unrelated to aneurysm rupture was also studied.

Clinical Material and Methods

Aneurysm Patients

The series included 19 patients (14 women and five men) who ranged in age from 29 to 68 years (mean age 51 years). The clinical data are summarized in Table 1. The patients were selected in random order from an ongoing study of immunogenetic markers in aneurysm patients.26 In each case the diagnosis was confirmed by computerized tomography (CT) scanning and preoperative cerebral angiography (Table 1). The amount of blood in the subarachnoid space was estimated from CT scans obtained at 11 different loci and was graded from 0 to 33 according to the scoring system of Ljunggren, et al.22 Patients with a large hematoma operated on within the first 24 hours after onset of symptoms were excluded. Since vasospasm usually occurs after
TABLE 1
Clinical data and levels of CIC in 19 patients with aneurysm rupture*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex, Age (yrs)</th>
<th>Associated Disorders</th>
<th>Site of Aneurysm</th>
<th>Clinical Grade on Admission†</th>
<th>CIC on Admission‡</th>
<th>Outcome at 3 Mos§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F, 36</td>
<td></td>
<td>lt ICA</td>
<td>I (Day 9)</td>
<td>0</td>
<td>recovered</td>
</tr>
<tr>
<td>2</td>
<td>F, 61</td>
<td>hypertension</td>
<td>lt ICA</td>
<td>IV (Day 2)</td>
<td>+++</td>
<td>dead (Day 14)</td>
</tr>
<tr>
<td>3</td>
<td>F, 68</td>
<td>hypertension</td>
<td>rt ICA</td>
<td>IV (Day 2)</td>
<td>+++</td>
<td>dead (Day 28)</td>
</tr>
<tr>
<td>4</td>
<td>F, 44</td>
<td>hypertension, stroke</td>
<td>basilar</td>
<td>III (Day 1)</td>
<td>+</td>
<td>dead (Day 8)</td>
</tr>
<tr>
<td>5</td>
<td>F, 57</td>
<td>hypertension</td>
<td>rt ICA</td>
<td>II (Day 2)</td>
<td>0</td>
<td>recovered</td>
</tr>
<tr>
<td>6</td>
<td>F, 54</td>
<td></td>
<td>lt ICA</td>
<td>II (Day 1)</td>
<td>+</td>
<td>moderately disabled</td>
</tr>
<tr>
<td>7</td>
<td>M, 67</td>
<td></td>
<td>ACA</td>
<td>III (Day 2)</td>
<td>0</td>
<td>recovered</td>
</tr>
<tr>
<td>8</td>
<td>M, 37</td>
<td></td>
<td>rt MCA</td>
<td>III (Day 1)</td>
<td>+</td>
<td>recovered</td>
</tr>
<tr>
<td>9</td>
<td>M, 65</td>
<td></td>
<td>ACA</td>
<td>II (Day 2)</td>
<td>0</td>
<td>severely disabled</td>
</tr>
<tr>
<td>10</td>
<td>F, 36</td>
<td></td>
<td>rt vertebral</td>
<td>II (Day 1)</td>
<td>+</td>
<td>recovered</td>
</tr>
<tr>
<td>11</td>
<td>M, 36</td>
<td></td>
<td>rt MCA</td>
<td>IV (Day 2)</td>
<td>+</td>
<td>moderately disabled</td>
</tr>
<tr>
<td>12</td>
<td>F, 58</td>
<td>hypertension, stroke</td>
<td>ACA</td>
<td>II (Day 1)</td>
<td>+++</td>
<td>moderately disabled</td>
</tr>
<tr>
<td>13</td>
<td>F, 53</td>
<td></td>
<td>rt ACA</td>
<td>IV (Day 1)</td>
<td>+</td>
<td>moderately disabled</td>
</tr>
<tr>
<td>14</td>
<td>M, 49</td>
<td></td>
<td>ACA</td>
<td>III (Day 1)</td>
<td>+</td>
<td>moderately disabled</td>
</tr>
<tr>
<td>15</td>
<td>F, 62</td>
<td></td>
<td>rt ICA</td>
<td>II (Day 2)</td>
<td>NA</td>
<td>recovered</td>
</tr>
<tr>
<td>16</td>
<td>F, 46</td>
<td>hypertension, stroke</td>
<td>lt ICA</td>
<td>IV (Day 2)</td>
<td>++</td>
<td>dead (Day 5)</td>
</tr>
<tr>
<td>17</td>
<td>F, 62</td>
<td></td>
<td>rt ICA</td>
<td>II (Day 2)</td>
<td>+</td>
<td>severely disabled</td>
</tr>
<tr>
<td>18</td>
<td>F, 29</td>
<td></td>
<td>rt ICA</td>
<td>II (Day 3)</td>
<td>0</td>
<td>recovered</td>
</tr>
<tr>
<td>19</td>
<td>F, 46</td>
<td></td>
<td>basilar</td>
<td>II (Day 2)</td>
<td>+++</td>
<td>dead (Day 19)</td>
</tr>
</tbody>
</table>

* CIC = circulating immune complexes; ICA = internal carotid artery; ACA = anterior communicating artery; MCA = middle cerebral artery; ACA = anterior cerebral artery.
† Grading according to Hunt and Hess.17
‡ For grading system of CIC see text. NA = results not available.
§ Outcome determined by the Glasgow Outcome Scale.18
‖ Indicates the aneurysm that ruptured in cases with more than one.

the 3rd day following SAH,19,21,33 the presence of cerebral vasospasm was studied in 14 patients who underwent angiography after that day (Table 2). Spasm was considered slight if only one major artery was narrowed or if the spasm was focal, moderate if two or three ipsilateral major arteries were affected, and severe if all major intracranial arteries were narrowed. The angiograms were all evaluated by a single observer (T.M.) who had no knowledge of the results of CIC and C3d measurements. The patients' clinical status was graded according to the classification of Hunt and Hess.17

Hematoma Patients

A group of seven patients admitted consecutively during the study period for treatment of intracerebral hematoma unrelated to aneurysm rupture was also investigated (Table 3). The mean age in this group was 53 years (range 18 to 66 years). The diagnosis was confirmed by CT scanning and in two cases also by angiography. The patients' clinical status was graded according to the classification of Hunt and Hess.17 Their functional capacity at the 3-month follow-up evaluation was assessed according to the Glasgow Outcome Scale,18 which was also used to evaluate the outcome of the aneurysm patients (see Table 1).

Blood Sampling Procedure

Blood samples were obtained within the first 24 hours after bleeding (Day 1), and also on Days 2, 3, 7, 10, and 14. Plasma (ethylenediaminetetra-acetic acid (EDTA) in a final concentration of 10 mM/liter) or serum was separated after 90 minutes at room temperature and stored at −80°C until analyzed.
Immune complexes and complement activation following SAH

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex, Age (yrs)</th>
<th>Associated Disorders</th>
<th>CT Findings</th>
<th>CIC on Admission†</th>
<th>Outcome at 3 Mos‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>M, 58</td>
<td></td>
<td>ICH, no cisternal blood, no blood in ventricle</td>
<td>++++</td>
<td>recovered</td>
</tr>
<tr>
<td>21</td>
<td>F, 18</td>
<td>epilepsy, AVM</td>
<td>ICH, no cisternal blood, no blood in ventricle</td>
<td>0</td>
<td>severely disabled</td>
</tr>
<tr>
<td>22</td>
<td>M, 54</td>
<td>hypertension</td>
<td>ICH with ventricular blood</td>
<td>0</td>
<td>severely disabled</td>
</tr>
<tr>
<td>23</td>
<td>M, 57</td>
<td>hypertension, stroke</td>
<td>ICH, no cisternal blood, no blood in ventricle</td>
<td>++++</td>
<td>dead (Day 32)</td>
</tr>
<tr>
<td>24</td>
<td>F, 52</td>
<td>hypertension</td>
<td>ICH, no cisternal blood, no blood in ventricle</td>
<td>0</td>
<td>moderately disabled</td>
</tr>
<tr>
<td>25</td>
<td>F, 66</td>
<td>claudication</td>
<td>ICH, no cisternal blood, no blood in ventricle</td>
<td>0</td>
<td>recovered</td>
</tr>
<tr>
<td>26</td>
<td>F, 66</td>
<td>hypertension, aortic aneurysm</td>
<td>ICH with ventricular blood</td>
<td>++++</td>
<td>dead (Day 45)</td>
</tr>
</tbody>
</table>

* CT = computerized tomography; ICH = intracerebral hematoma; AVM = arteriovenous malformation.
† For grading of circulating immune complexes (CIC) see text.‡ Outcome determined by the Glasgow Outcome Scale.

Assay for Detection of CIC

The polyethylene glycol (PEG)-complement consumption assay was used to detect CIC. This assay has previously been described in detail. Briefly, the plasma and serum are each subjected to PEG precipitation followed by washings in PEG-6000 in borate buffer, pH 8.3 (final concentration of PEG 2.75% and 3.5%, respectively, for each of two serum assays), and the precipitate is resuspended in 0.15 M veronal buffer, pH 7.4, and mixed with guinea pig serum absorbed with sheep erythrocytes. The amount of guinea pig complement was adjusted to achieve optimal sensitivity in each experiment. Complement consumption is measured as a reduction of hemolytic capacity after the addition of sensitized sheep erythrocytes. The anti-complementary activity of each specimen, measured as inhibition of hemolysis, was expressed in arbitrary units from 0 (not detectable) to ++++ (high activity). Positivity for CIC was defined as the mean of control measurements ± 2 standard deviations (SD).

Quantification of C3d

The presence of C3d in EDTA-plasma was quantified by rocket immunoelectrophoresis using the intermediate gel technique as described in detail previously.

This technique was performed in 1% modified Litex LSA agarose in 0.028 M veronal buffer, pH 8.6. In order to avoid a pH gradient across the agarose during electrophoresis, double chambers were used on both the anode and cathode sites. The electrophoresis was run at 7.5 V/cm for 7 hours. The C3d concentration was corrected for changes in hemoconcentration. Normal values of C3d in 21 age- and sex-matched healthy blood donors ranged from 11 to 50 mU/ml (mean 22.2 mU/ml).

Statistical Analysis

The chi-square test, Fisher’s exact test, Student’s t-test for unpaired differences, the Wilcoxon-Mann-Whitney test, and analysis of variance (ANOVA) were used for statistical analysis. A p value less than 0.05 was considered significant.

Results

Clinical Findings

As shown in Table 1, five of the 19 aneurysm patients died. Seven patients were moderately or severely disabled at 3 months, and seven patients had recovered completely. Vasospasm was demonstrated in nine of the 14 patients who underwent angiography between Days 4 and 12 after SAH (Table 2). Due to late admission on Day 9, one patient (Case 1) was not included in the CT score calculations. The remaining 18 patients had a mean subarachnoid bleeding score on admission of 20.5 ± 9.3 SD (range 2 to 31). There was no difference in CT score between patients with and without vasospasm.

Two of the hematoma patients died, three were disabled, and two recovered completely (Table 3).

Circulating Immune Complexes

Patients with Ruptured Aneurysm. At admission, CIC were detected in 13 (72%) of 18 patients with ruptured aneurysm (Table 1) as compared to three of 21 age- and sex-matched healthy blood donors (χ² = 11.2, p < 0.001). Four of the five patients who were negative for CIC at admission remained so throughout the study period, whereas one patient (Case 18) became positive on Day 14. Table 1 shows the relationship between some clinical parameters and CIC at admission. Of the five patients without detectable CIC, one was in Hunt and Hess Grade III while the remaining four were in good clinical condition (Grade I or II). Four of these five had recovered fully by 3 months. In contrast, eight of the 13 aneurysm patients who were positive for CIC were in Grade III or IV at admission, and at the end of the 3-month follow-up period only two had recovered fully (Fisher’s exact test, p < 0.05).
Five patients with CIC died, whereas none of the CIC-negative patients died. The relationship between CIC and vasospasm is shown in Table 2. Eight of nine patients with spasm had CIC on admission, while CIC were present on admission in one of four patients without vasospasm.

Patients with Intracerebral Hematoma. Three of the seven patients with intracerebral hematoma had CIC on admission (Table 3). These three patients remained CIC-positive throughout the study period, whereas two patients (Cases 22 and 25) became CIC-positive from Day 7 and Day 10, respectively, following cerebral hemorrhage. In this group, no association could be demonstrated between the outcome of the disease and the presence of CIC.

Complement Activation

Table 4 shows the mean plasma levels of C3d during the study period in the 19 aneurysm patients and in the seven patients with cerebral hematoma unrelated to aneurysm rupture. The C3d levels on Days 1 to 3 following the onset of symptoms in the different patient groups did not differ from those of apparently healthy blood donors as determined by Student's t-test. On Days 7, 10, and 14, the C3d levels in aneurysm patients were significantly higher than those in blood donors but were not significantly higher than those in the hematoma patients (Student's t-test).

The levels of complement C3d in the 14 aneurysm patients investigated angiographically for vasospasm later than Day 3 after SAH are depicted in Fig. 1. In the patients with angiographic vasospasm, there was a marked and sustained increase in the plasma C3d concentration after Day 3. In patients without spasm, the C3d levels remained close to those of the healthy donors. Table 5 compares the mean plasma C3d levels ± SD from Days 7, 10, and 14 in aneurysm patients with spasm (nine patients, Group 1) and without spasm (five patients, Group 2), in seven hematoma patients (Group 3), and in 21 blood donors (Group 4). As can be seen, the aneurysm patients with cerebral vasospasm (Group 1) had significantly higher C3d levels than any of the other three groups in this period (that is, during the 2nd week after onset of symptoms). A positive association was found between the presence of CIC on admission and the mean C3d levels on Days 7, 10, and 14, as well as between the presence of CIC on admission and the mean increase in C3d concentration from the first 3 days following SAH to the 2nd week (Fig. 2). There was no correlation between CT score on admission and the C3d levels in the 2nd week after onset of symptoms (Fig. 3).

Two of the hematoma patients developed severe breakthrough bleeding into the ventricles (Table 3). One of them (Case 26) had CIC on admission and showed an increase in her C3d level from 27.3 mU/ml (mean value for Days 1, 2, and 3) to 38 mU/ml (mean value for Days 7, 10, and 14). No change in C3d level was found in the other patient (Case 22), who had no CIC on admission.

Discussion

This study demonstrates that the majority of patients with ruptured cerebral aneurysms have CIC on admission to the hospital. The CIC in our series of patients were associated with the development of angiographic
Immune complexes and complement activation following SAH

vasospasm, a poor prognosis, and complement activation.

A high incidence of CIC in aneurysm patients with angiographic and/or clinical vasospasm has previously been reported.27 In that study, however, only one blood sample was obtained from each patient and the sample was drawn at different times following the SAH. Thus, the time course for the formation of the CIC and the possible pathophysiological role of CIC in the development of the spasm were not elucidated. Our study confirms that patients with SAH from ruptured aneurysms have a high incidence of CIC. In this context it should be emphasized that the prevalence of CIC in our control blood donors is close to that found by others.27 Moreover, our investigation showed that the CIC were present at admission to the hospital, which for some patients occurred within the first 10 hours after SAH. The CIC must therefore have been formed either very shortly after the hemorrhage or even before the aneurysm ruptured, and thus were present before spasm usually developed.14,19,21,33

A high incidence of CIC has previously been found in patients with various vascular disorders,8 particularly in patients with acute myocardial infarction.7,9,28 In the present study, two of the three CIC-positive hematoma patients were hypertensive and had previously suffered a vascular accident. Of the 13 CIC-positive aneurysm patients, five were hypertensive and three of these had previously suffered a major cerebral stroke. However, since CIC were present in eight of 12 aneurysm patients who neither suffered from hypertension nor had experienced a vascular event, the presence of CIC in aneurysm patients cannot be solely ascribed to such disorders. On the other hand, the majority of aneurysm patients admitted to the hospital experience warning symptoms days to months before a major hemorrhage occurs.6,10,20,25,32 The early occurrence of CIC in aneurysm patients may therefore be explained by the repeated leakage of small amounts of blood into the subarachnoid space with concomitant release of damaged tissue, which may act as an autoantigen. The specimens remained CIC-positive throughout the 2-week study period, and one aneurysm patient and two hematoma patients became CIC-positive during the

| TABLE 5 |

| Mean plasma levels (mU/ml) of C3d from Days 7, 10, and 14 after onset of symptoms in four groups of patients* |
|-------------------------|------------------|------------------|------------------|------------------|
| Value                  | Group 1 | Group 2 | Group 3 | Group 4 |
| mean                   | 41.9    | 20.6    | 27.5    | 22.2    |
| standard deviation     | ± 13.0  | ± 7.6   | ± 6.7   | ± 9.6   |

* For a description of the four groups see Results section. Significance was determined by Student's t-test: Group 1 vs. Group 2 = p < 0.005; Group 1 vs. Group 3 = p < 0.05; Group 1 vs. Group 4 = p < 0.0001. Comparisons of Group 2 vs. Group 3, Group 2 vs. Group 4, and Group 3 vs. Group 4 showed no significant difference.

**FIG. 2.** Relationship between circulating immune complexes (CIC) on admission and C3d levels in the 2nd week following subarachnoid hemorrhage (X C3d = mean value for Days 7, 10, 14), and the increase in C3d levels (Δ C3d = mean value for Days 7, 10, and 14 minus mean value for Days 1, 2, and 3). The difference was statistically significant at p < 0.05 and p < 0.01, respectively, according to the Wilcoxon-Mann-Whitney test.

**FIG. 3.** Relationship between the computerized tomography (CT) score on admission and C3d levels in the 2nd week following aneurysm rupture. No significant correlation was found. Filled circles indicate aneurysm patients with spasm; open circles indicate aneurysm patients without spasm. CIC = circulating immune complexes.
study. This could be explained by a continuous release of damaged tissue which maintained production of the CIC.

A pathogenetic role of CIC in disease depends on whether the CIC are deposited in tissues and whether they activate complement. Deposition of IgG and complement C3 in the cerebral arterial wall has previously been reported in patients with chronic vasospasm.

In the present study we found that patients who developed angiographic arterial narrowing, in contrast to patients who did not, exhibited a twofold increase in C3d levels, a sensitive marker for complement activation. Moreover, it was found that the increase in C3d was positively associated with the presence of CIC. Our findings thus suggest that the demonstrated CIC had complement-activating properties, but the plasma C3d may also have been generated locally due to an antigen-antibody reaction around the affected vessels.

The complement system can be regarded as a principal effector mechanism of inflammation in immune complex-mediated diseases. Inflammatory cellular reactions with accumulation of polymorphonuclear leukocytes in and around cerebral vessels following aneurysm rupture have repeatedly been reported. Complement has also been shown to solubilize immune complexes and thereby enhance their removal. The observed association between complement activation, CIC, and vasospasm may thus be either a primary or a secondary phenomenon. In the latter case, it should be expected that hematoma patients would also have shown an increase in the plasma concentration of C3d; however, this was not found. Moreover, no correlation could be demonstrated between the amounts of blood in the subarachnoid space and C3d. The present findings therefore suggest that immunological reactions are involved in the pathogenesis of cerebral vasospasm following aneurysm rupture. Further evidence of a possible involvement of immunological processes comes from the clinical observation that a rebleeding episode induces vasospasm earlier than does the primary hemorrhage. This may illustrate the rapid secondary "immune" response following a more slowly developing primary response. The findings support the concept that administration of high-dose methylprednisolone (30 mg/kg), which can be expected to inhibit complement-induced immune injuries, may be of benefit in aneurysm patients. Prevention of chronic vasospasm in a "double-hemorrhage" canine model has been obtained using this regimen.

Acknowledgments

The authors thank Mrs. Jette Brandt and Mrs. Lise Schröder for expert technical assistance.

References


Immune complexes and complement activation following SAH

864–873, 1984


Manuscript received June 27, 1986.
This work was supported by the Konventualinde Marie de Lancy Pedersens Foundation.
Address reprint requests to: John R. Østergaard, M.D., Department of Neurosurgery GS, Aarhus Kommunehospital, DK-8000 Aarhus C, Denmark.