Evidence of Intrathecal Immunoglobulin Synthesis in Stroke

A Cohort Study

Harald Prüss, MD; Deetje Iggena, MD; Tina Baldinger, PhD; Vincent Prinz, MD; Andreas Meisel, MD; Matthias Endres, MD; Ulrich Dirnagl, MD; Jan M. Schwab, MD, PhD

Background: Immune mechanisms are included in stroke pathophysiologic factors, but the frequency and role of intrathecal antibodies is unclear and diagnostic tests are not routinely performed on cerebrospinal fluid (CSF).

Objective: To determine the frequency of intrathecal immunoglobulin synthesis in a well-characterized cohort of patients who experienced “noninflammatory” acute stroke.

Design: Retrospective cohort study.

Setting: University hospital neurology department.

Patients: Patients (n=318) with stroke who were undergoing lumbar puncture during diagnostic workup and 79 control patients.

Results: Cerebrospinal fluid–specific immunoglobulin (IgG, IgM, and IgA) synthesis was significantly (P<.001) more frequent after stroke (24.8%) compared with the incidence in age- and sex-matched controls (2.5%). Furthermore, 31.3% of stroke patients demonstrated blood-brain barrier dysfunction and 18.1% displayed pleocytosis.

Conclusion: The strong association between CSF-specific immunoglobulin synthesis and stroke suggests a role in the development of cerebral ischemia and might constitute an immunologically defined stroke subgroup.


Immune mechanisms are being increasingly considered as factors associated with the development of cerebral ischemia.1,2 However, the roles of antibodies and B cells in stroke have been largely neglected, although the occasional finding of oligoclonal bands (OBs) in the cerebrospinal fluid (CSF) of patients who have experienced stroke has been reported3 for more than 40 years. Oligoclonal bands were often referred to as being unspecific, but some evidence suggests that OBs may result from a specific immune response after presentation of central nervous system (CNS) antigens to the immune system. For example, in patients with an acute first-ever stroke, the levels of antineurofilament antibodies were elevated for between 1 and 6 months following the stroke compared with initial levels, whereas antibodies against a ubiquitous antigen, cardiolipin, did not change significantly.4 These studies have several limitations, including the small number of patients5,6 or the lack of routine brain imaging in earlier studies. To provide a validated basis for CSF-specific OBs in the pathophysiologic factors associated with stroke, we analyzed a well-characterized large cohort of patients with acute stroke.

METHODS

PATIENTS

In a retrospective cohort study, 3050 consecutive patients with ischemic stroke hospitalized during a 5-year period (January 1, 2005, through December 31, 2009) at the
uncertainty. All patients underwent cerebral computed tomography or magnetic resonance imaging and lumbar puncture within 96 hours after symptom onset. After exclusion of inflammatory disease, consecutive age- and sex-matched patients who had received lumbar puncture during a diagnostic workup for headache (n = 24), diabetic ocularmotor or abducens nerve palsy (n = 16), idiopathic facial nerve palsy (n = 29), and dizziness (n = 10) were used as controls (n = 79).

**INTRATHECAL IMMUNOGLOBULIN SYNTHESIS**

Albumin, immunoglobulin (Ig) G, IgA, and IgM from CSF and serum samples were quantified by routine nephelometry. Blood-brain barrier dysfunction was determined on the basis of age-related albumin quotients of CSF/serum. For detection of intrathecal antibody synthesis, the antibody index was calculated as the ratio between the CSF/serum quotient for IgG, IgM, and IgA antibodies and the CSF/serum albumin quotient using the Reibergram calculation. Oligoclonal bands were detected by isoelectric focusing with silver stain. Participation in the quarterly German quality-control survey for the detection of OBs was significantly different from that of the 79 control patients.

**RESULTS**

All 318 patients with stroke had radiologically confirmed cerebral ischemia and exclusion of CNS infection (eg, neurotropic viruses, *Borrelia* serologic findings, and antinuclear antibodies), demyelinating disease, head trauma, and intracerebral neoplasm. Age and sex data were not significantly different from those in the control group (Figure, A; Mann-Whitney and Fisher exact tests used for analysis of age and sex, respectively). The CSF cell counts ranged from 0 to 120/µL (mean [SD], 4.7 [13.3]/µL); 18.1% had pleocytosis (CSF cell count, ≥5/µL). Protein concentration ranged from 9.7 to 363.4 mg/dL (mean [SD], 64.6 [51.0] mg/dL); 32.9% had increased CSF protein (>45 mg/dL), and 33.1% had blood-brain barrier dysfunction. Intrathecal immunoglobulin synthesis in the CSF compartment was present in 24.8% of patients with stroke: 17.9% revealing CSF-specific oligoclonal IgG bands using isoelectric focusing (several also with increased IgG antibody indices) and 6.9% showing increased CSF/serum antibody indices for IgM and IgA. Representative images show multiple strong OBs in selected patients with stroke (Figure, B). The frequency of OBs was significantly different from that of the 79 control patients without CNS disease, of whom only 2.5% had OBs (P < .001, Fisher exact test), and none of the control patients had pleocytosis.

In contrast to the population in a small study (N = 16), stroke patients with OBs were not significantly different in age from those without OBs (P = .87, Mann-Whitney 2-tailed test). Also, there was no association between the presence of OBs and sex, type of ischemia, frequency of pleocytosis, or blood-brain barrier dysfunction (Table). Pleocytosis was lymphocyte-dominant in both groups, with significantly fewer macrophages in patients with OB-positive stroke. Relevant previous illnesses (eg, tumor, rheumatoid disease) or current systemic infections were not significantly more frequent in patients with OB-positive stroke (Table).

**COMMENT**

Intrathecal immunoglobulin synthesis was determined by the presence of oligoclonal IgG bands and of IgM and IgA antibody serum to CSF indices and was present in 24.8% of stroke patients in whom no infectious or autoimmune cause was identified during clinical workup. This unexpectedly high prevalence of OBs in this population may point to a direct association between CSF-specific immunoglobulin synthesis and focal cerebral ischemia. Because of the invasive nature of lumbar puncture, CSF samples from healthy individuals are not available for comparison. Our control group without any evident CNS disease had a low OB frequency of 2.5%, which is likely in the range of healthy individuals. Indeed, published studies on noninflammatory cohorts of between 134 and 207 patients (eg, having disk prolapse, headache, or dizziness) revealed OBs in none to 3.9% of the population.
In previous reports on cerebrovascular disease, limited by small sample size, the percentage of OBs varied widely between 7 of 14 patients (50%) with stroke identified using isoelectric focusing and 4 of 85 patients (5%) with acute cerebrovascular disease identified using agar gel electrophoresis. Oligoclonal bands were detected in 10 patients with stroke of another study, and 23 patients with infarct had higher CSF IgG levels compared with healthy control participants.

Diagnostic tests using CSF are not routinely performed in patients after cerebral ischemia events. In our cohort, additional symptoms (although not uncommon in stroke, eg, agitation, disorientation, and seizure) led to lumbar puncture to rule out inflammatory CNS disease. We cannot exclude the possibility that this selection enriched the retrospective cohort with more severely ill stroke patients.

Stroke-associated intrathecal immunoglobulin synthesis may result from (1) an underlying unidentified inflammatory disease, (2) undetected previous ischemic degeneration of neuronal tissue with repeated presentation of CNS antigen to the immune system, or (3) polyclonal nonspecific B-cell activation secondary to brain damage. The second explanation might be relevant to the high proportion of patients with OBs already present at the time of their first clinically detected stroke. The finding of OBs in patients with transient ischemic attacks supports this notion and implies relevance for predisease stages.

Because of the retrospective study design, it is unclear whether stroke-related intrathecal immunoglobulins represent specific antibody-mediated autoreactivity and whether this is relevant for pathologic factors that lead to stroke and clinical outcome (eg, determined with the use of the National Institutes of Health Stroke Scale). In a rodent study, induction of anti-neurofilament antibodies was associated with cognitive deficits. Similarly, our findings stimulate the question whether the high frequency of poststroke dementia (30%, often with atrophy) is associated with intrathecal immunoglobulin synthesis. Along these lines, it is tempting to speculate whether the recently defined CNS injury–induced immune depression syndrome might suppress overt humoral and cellular autoreactivity after CNS injury such as stroke.

We conclude that the unexpectedly high prevalence of intrathecal immunoglobulin synthesis in patients with stroke demands a systematic prospective analysis of CSF and serum samples to determine the time kinetics and pathogenicity of antibodies. Future experiments should evaluate antigen specificity and the relation to cellular immunity after stroke.

### Table. Characteristics of Patients With Ischemic Stroke

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Intrathecal Immunoglobulin Synthesis</th>
<th>No Intrathecal Immunoglobulin Synthesis</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>58.1 (14.9)</td>
<td>57.6 (16.0)</td>
<td>.22</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>55.7</td>
<td>47.7</td>
<td>.24</td>
</tr>
<tr>
<td>Female</td>
<td>44.3</td>
<td>52.3</td>
<td>.24</td>
</tr>
<tr>
<td>Pleocytosis, ≥5 WBC/µL</td>
<td>21.5</td>
<td>17.1</td>
<td>.40</td>
</tr>
<tr>
<td>BBB dysfunction</td>
<td>39.2</td>
<td>28.6</td>
<td>.28</td>
</tr>
<tr>
<td>Territorial infarction</td>
<td>87.3</td>
<td>80.9</td>
<td>.64</td>
</tr>
<tr>
<td>Lacunar infarction</td>
<td>7.6</td>
<td>5.8</td>
<td>.59</td>
</tr>
<tr>
<td>Lymphocytes, mean (SD), %</td>
<td>68.2 (27.9)</td>
<td>64.4 (22.7)</td>
<td>.47</td>
</tr>
<tr>
<td>Macrophages, mean (SD), %</td>
<td>13.4 (8.8)</td>
<td>21.3 (14.9)</td>
<td>.01</td>
</tr>
<tr>
<td>Neutrophils, mean (SD), %</td>
<td>15.9 (29.4)</td>
<td>11.7 (22.0)</td>
<td>.42</td>
</tr>
<tr>
<td>History of tumor</td>
<td>7.3</td>
<td>13.2</td>
<td>.23</td>
</tr>
<tr>
<td>History of rheumatoid diseases (eg, SLE, APS, Sjögren syndrome)</td>
<td>9.8</td>
<td>9.6</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Systemic infection (eg, sinusitis, urinary tract infection, gastritis)</td>
<td>9.8</td>
<td>10.1</td>
<td>&gt;.99</td>
</tr>
</tbody>
</table>

Abbreviations: APS, antiphospholipid syndrome; BBB, blood-brain barrier; SLE, systemic lupus erythematosus; WBC, white blood cell.

aPoststroke cerebrospinal fluid cells were primarily lymphocytes.

bFisher exact test or Mann-Whitney test, 2-tailed, used in analysis.
REFERENCES