Severe Stroke Induces Long-Lasting Alterations of High-Mobility Group Box 1

Juliane Schulze, BS; Dannielle Zierath, BS; Patricia Tanzi, BSN, RN, CCRC; Kevin Cain, PhD; Dean Shibata, MD; Alexander Dressel, MD; Kyra Becker, MD

Background and Purpose—The signals that initiate the poststroke inflammatory response are unknown. High-mobility group box (HMGB) 1 protein is a nuclear protein that is passively released from necrotic tissue and is able to activate leukocytes, which in turn secrete HMGB1. HMGB1 is also able to activate antigen-presenting cells and therefore stands at the crossroads of innate and adaptive immunity.

Methods—Plasma HMGB1 concentrations were determined at multiple time points after ischemic stroke (N=110) and correlated to stroke severity and biomarkers of inflammation. The relationships between HMGB1, stroke outcome, and autoimmune responses to brain antigens were also assessed.

Results—Stroke resulted in an increase in HMGB1 that persisted for 30 days. Plasma HMGB1 was correlated with the number of circulating leukocytes but was not predictive of either stroke outcome or the development of autoimmune responses to brain antigens. Patients with a T1(+) response to myelin basic protein at 90 days after stroke, however, had higher plasma HMGB1.

Conclusions—HMGB1 appears to be involved in the postischemic inflammatory response, but it remains unclear whether HMGB1 initiates this response or merely reflects activation of leukocytes by another signal. (Stroke. 2013;44:246-248.)

Key Words: alarmin ■ DAMP ■ HMGB1 ■ inflammation ■ monocytes ■ stroke

Stroke induces complex changes in the immune response, leading to systemic inflammation as well as impaired host defense.1-4 Both the degree of inflammation and the degree of host response impairment are related to stroke severity and infarct volume.1,2,4 The dysfunction in host defense is mediated by the sympathetic nervous system; the signals that initiate systemic inflammation are unknown.3

The poststroke systemic inflammatory response is not directed in an antigen-specific fashion. Systemic infections that activate the innate immune response, however, increase the likelihood of T1-type immune responses to brain antigens in patients with stroke.5 The link between infection and the development of central nervous system autoimmunity may be mediated by danger-associated molecular patterns, which are derived from pathogens or released from host cells.6 High-mobility group box (HMGB) 1 is a ubiquitous nuclear protein released from necrotic cells and secreted by activated leukocytes.7 Once released, HMGB1 functions as a danger-associated molecular pattern by activating antigen-presenting cells through toll-like receptors, and the receptor for advanced glycation end-products.6

Neutralizing HMGB1 improves outcome in experimental stroke.8,9 The relationship between plasma HMGB1 and clinical stroke outcome is unknown. In this study we investigate whether plasma HMGB1 concentrations in patients (1) reflect infarct size, (2) promote T1(+) responses to brain antigens, or (3) are predictive of stroke outcome.

Materials and Methods

Research Subjects
The patient population for this study is described elsewhere.5 Patients with acute ischemic stroke were enrolled as soon as possible after stroke onset. Blood was drawn at 24 hours (±6 hours; N=38), 72 hours (±12 hours; N=98), 7 days (±1 day; N=94), 30 days (±5 days; N=89), 90 days (±5 days; N=72), 180 days (±5 days; N=70), and 365 days (±5 days; N=24) after stroke. The study was approved by the Institutional Review Board. Patients or their surrogates provided informed consent.

Clinical Data
Stroke severity was determined by the National Institutes of Health Stroke Scale, and outcome by the modified Rankin Scale. Infarct volume on initial diffusion-weighted magnetic resonance imaging was calculated by the ABC/2 method.10

Laboratory Studies
Leukocyte counts and concentrations of C-reactive protein were determined by hospital clinical laboratories. Additional plasma was immediately frozen at −80°C, and HMGB1 concentrations determined by enzyme-linked immunoassay (IBL International); the sensitivity of the assay was 0.20 ng/mL. Isolated lymphocytes were isolated and frozen in liquid nitrogen until use.
T helper (Th1) responses to lymphocytes were determined, as described elsewhere.\(^5\)

**Statistics**

Descriptive data are presented as median and interquartile range; group comparisons were performed using the Kruskal-Wallis H test or the Mann–Whitney U test. Correlations are presented as either Pearson \(r\) or Spearman \(\rho\). Logistic regression was used to assess the contribution of HMGB1, C-reactive protein, and leukocyte subsets to poor outcome (modified Rankin Scale >3) at 90 days after stroke, and to the risk of developing a Th1(+) response to myelin basic protein (MBP). Significance was set at \(P<0.05\).

**Results**

A total of 114 patients were enrolled in the parent study; baseline characteristics are described elsewhere.\(^5\) Plasma HMGB1 was available for 110 of these patients, who are the subject of this report. At day 3 after stroke, there were weak correlations between HMGB1, infarct volume (\(r=0.217, P=0.024\)), and stroke severity (\(\rho=0.230, P=0.015\)). Plasma HMGB1 and CRP were highest in patients with severe strokes (National Institutes of Health Stroke Scale \(\geq 17\)), and remained elevated for months (Figure).

Neither the number of leukocytes nor the plasma concentrations of HMGB1 early after stroke were independently predictive of stroke outcome at 90 days (Table 1). Higher concentrations of CRP early after stroke, however, were associated with worse 90 day outcomes. The number of leukocytes was highly correlated (independent of infarct volume) to plasma HMGB1 throughout the study period: \(r=0.415, P=0.415\) at day 1; \(r=0.312, P=0.002\) at day 3; \(r=0.0297, P=0.004\) at week 1; \(r=0.374, P<0.001\) at month 1; \(r=0.475, P<0.001\) at month 3; and \(r=0.539, P=0.010\) at year 1. The relationship between CRP and HMGB1 was more variable.

Among patients with a Th1(+) response to MBP at 90 days,\(^5\) plasma HMGB1 and CRP were also elevated at that time point (Table 2). There was, however, no relationship between HMGB1 concentrations early after stroke onset and the propensity to develop a Th1(+) response to MBP at 90 days.

**Discussion**

A systemic inflammatory response is common after stroke. Alarmins like HMGB1 are candidate molecules that could initiate the innate immune response following tissue damage.\(^11,12\)

**Table 1.** Predictive Value of Early (Day 3) Markers of Inflammation on Poor Outcome (mRS>3) at 90 Days After Stroke

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted for NIHSS</th>
<th>Adjusted for NIHSS and Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>(P) Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>90 days (N=102)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes (per thou/μL)</td>
<td>1.183 (1.047–1.336)</td>
<td>0.007</td>
<td>1.035 (0.884–1.212)</td>
</tr>
<tr>
<td>Neutrophils (per thou/μL)</td>
<td>1.367 (1.138–1.641)</td>
<td>0.001</td>
<td>1.120 (0.884–1.418)</td>
</tr>
<tr>
<td>Lymphocytes* (per thou/μL)</td>
<td>0.181 (0.052–0.638)</td>
<td>0.008</td>
<td>0.518 (0.132–2.031)</td>
</tr>
<tr>
<td>Monocytes (per thou/μL)</td>
<td>41.40 (5.481–312.8)</td>
<td>&lt;0.001</td>
<td>7.490 (0.527–106.5)</td>
</tr>
<tr>
<td>HMGB1 (per ng)</td>
<td>0.998 (0.939–1.061)</td>
<td>NS</td>
<td>0.960 (0.882–1.044)</td>
</tr>
<tr>
<td>CRP (per 10 mg/L)</td>
<td>1.311 (1.160–1.482)</td>
<td>&lt;0.001</td>
<td>1.166 (1.026–1.325)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; CRP, C-reactive protein; HMGB, high-mobility group box; mRS indicates modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; NS, not significant; OR, odds ratio; and thou/μL, thousand per μL.

*All values represent the highest recorded value within the first 3 days except for lymphocytes, where the lowest recorded value was used.
Activated leukocytes are also a source of HMGB, and the initiation of inflammation or activating antigen-presenting cells through toll-like receptors and advanced glycation end products, we expected that high concentrations of plasma HMGB1 early after stroke onset would be predictive of poor outcome and predispose to autoimmune responses to brain antigens, yet plasma HMGB1 was only weakly associated with infarct volume.\textsuperscript{13} Activated leukocytes are also a source of HMGB, and the robust association between HMGB1 and the leukocyte numbers suggest that immune cells might be the primary source of plasma HMGB1 following stroke.

Given its ability to promote inflammation and activate antigen-presenting cells through toll-like receptors and advanced glycation end products, we expected that high concentrations of plasma HMGB1 early after stroke onset would be predictive of poor outcome and predispose to autoimmune responses to brain antigens, yet plasma HMGB1 was predictive of neither. The lack of an association between HMGB1, infarct size, and autoimmune responses to brain antigens suggests that HMGB1 is not the single factor initiating inflammation or activating antigen-presenting cells after stroke. At day 90 after stroke, however, those patients with a T\textsuperscript{1} (+) response to MBP had increased plasma HMGB1; the source of this HMGB1 is unknown.

In summary, plasma HMGB1 is elevated following ischemic stroke; patients with severe stroke have higher HMGB1, and these elevations last for months. The correlation between plasma HMGB1 and leukocyte numbers is more robust than that between plasma HMGB1 and infarct volume, suggesting that plasma HMGB1 reflects secretion by leukocytes. Finally, HMGB1 did not predict stroke outcome or development of autoimmune responses to MBP. Further studies are needed to define the role of HMGB1 in poststroke inflammation.

### Sources of Funding
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### Disclosure
None.

### References

### Table 2. Differences in Inflammatory Markers Between Patients With a Th1(+) Response to MBP at 90 Days and Those Without

<table>
<thead>
<tr>
<th>90 days</th>
<th>Yes (N=19)</th>
<th>No (N=47)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (thou/μL)</td>
<td>7.73 (5.94, 9.41)</td>
<td>6.94 (5.39, 7.99)</td>
<td>0.111</td>
</tr>
<tr>
<td>Neutrophils (thou/μL)</td>
<td>4.44 (3.36, 6.51)</td>
<td>4.01 (3.09, 5.29)</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes (thou/μL)</td>
<td>1.75 (1.37, 2.14)</td>
<td>1.73 (1.33, 2.14)</td>
<td>NS</td>
</tr>
<tr>
<td>Monocytes (thou/μL)</td>
<td>0.57 (0.44, 0.66)</td>
<td>0.50 (0.41, 0.66)</td>
<td>NS</td>
</tr>
<tr>
<td>HMGB1 (ng/mL)</td>
<td>5.70 (2.51, 8.11)</td>
<td>3.27 (2.12, 4.49)</td>
<td>0.030</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>8.10 (0.80, 21.1)</td>
<td>1.90 (0.60, 3.92)</td>
<td>0.036</td>
</tr>
</tbody>
</table>

T\textsuperscript{1}(+) response to MBP is a response greater than that seen in 75% of the control population.\textsuperscript{1} CRP indicates C-reactive protein; HMGB, high-mobility group box; and NS, not significant.
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