Analysis of Lymphocyte Subsets in Patients With Stroke and Their Influence on Infection After Stroke

Antje Vogelgesang, MSc; Uwe Grunwald, MD; Sönke Langner, MD; Robert Jack, PhD; Barbara M. Bröker, MD; Christof Kessler, MD; Alexander Dressel, MD

Background and Purpose—Recent studies have attributed the increased infection vulnerability of patients with stroke to stroke-induced immunosuppression. We have therefore explored the immunological changes in patients with ischemic stroke.

Methods—Blood from 46 patients with stroke was analyzed by fluorescent-activated cell sorter to determine leukocyte subsets. To identify changes that represent clinically relevant immunosuppression, we compared patients who developed infection within 14 days after stroke with those who did not.

Results—Stroke induced a dramatic and immediate loss of T-lymphocytes, most pronounced within 12 hours after stroke onset. Only patients with subsequent infection exhibited a delay in the recovery of CD4⁺ T-lymphocyte counts.

Conclusions—Our data suggest that a loss of CD4⁺ T cell function contributes to the stroke-induced immunosuppression. The CD4⁺ T cell count on the day after stroke may emerge as a predictive marker for poststroke infection allowing, early identification of patients at risk. (Stroke. 2008;39:237-241.)

Key Words: immunology | infection | ischemia | stroke

The increased infection vulnerability of patients with stroke has recently been attributed to stroke-induced immunosuppression. Currently, the strongest support for this hypothesis comes from a mouse model of ischemic stroke demonstrating that affected animals develop lymphocytopenia and spontaneous infection.

The present study analyzes immunological changes caused by stroke in humans and identifies potential prognostic markers that may allow selection of patients prone to infection.

Materials and Methods

Patients and Control Subjects
Forty-six patients with onset of stroke symptoms less than 12 hours before admission to the stroke unit were recruited into the study and compared with 14 age-matched nonstroke control subjects (Table). Patients with clinical signs of infection on admission were not recruited. Ischemic stroke was diagnosed clinically and by cerebral CT. Blood was obtained from patients immediately on admission and between 6:00 AM and 7:00 AM on days 1, 7, and 14 thereafter.

For the purpose of this study, we defined the following criteria of infection: (1) presence of clinical signs of infection (pneumonia, urinary tract infections, fever of unknown origin); (2) serum concentrations of C-reactive protein >0.5 ng/mL; and (3) procalcitonin serum concentrations >0.5 ng/mL. To compare patients who developed infection after stroke with those who did not, 2 cohorts were formed. In the infected cohort, all 3 criteria for infection had to be fulfilled on day 7 or 14. In the noninfected cohort, none of the criteria was matched throughout the whole study period. Patients who matched some criteria but not all were not assigned to either cohort.

The study protocol was approved by the local ethics committee. All patients gave fully informed consent directly or through a surrogate when appropriate.

Routine cerebral CT images were acquired on a 16-row multislice CT scanner (Somatom 16; SIEMENS Medical Systems). To calculate lesion size, images were analyzed using OSIRIX 2.5.1.

Cell types were defined by surface marker expression determined in a fluorescence-activated cell sorter analysis performed on a FACScalibur (Beckton Dickinson, Heidelberg, Germany) using True-Count tubes, Simulset IMK Plus-reagents, fluorescently labeled antibodies to CD3 (Peridiniumchlorophyll–Protein [PerCP]), CD45 (PerCP), HLA-DR (fluorescein isothiocyanate conjugated; BD Biosciences) and CD5 (fluorescein isothiocyanate conjugated), CD14 (Allo–Phyco–Cyanin [APC]), CD19 (Phycoerythrin [PE]; Caltag, Hamburg, Germany).

Serum Protein Concentrations
Procalcitonin serum concentration was measured on a Liaison analyzer (DiaSorin, Dietzenbach, Germany; reagents: Brahms, Hennigsdorf, Germany); C-reactive protein on a Dimension Rxl (Dade Behring, Eschborn, Germany); and interleukin-6 on an Immulite 2500 (DPC Biermann, Bad Nauheim, Germany).

Student t test or one-way analysis of variance was applied as appropriate using Graph Pad Prism 3.02 (GraphPad Software, San Diego, Calif). Logistic regression analysis was performed using SPSS 15 (SPSS Inc, Chicago, Ill).
Results

Stroke Induces Rapid Immunological Changes in Peripheral Blood

On admission, leukocyte counts were slightly elevated due to an increased number of granulocytes (Figure 1A–B). Absolute lymphocyte counts were reduced in comparison to control individuals (Figure 1D). There was a dramatic loss of CD4+ and CD8+ T cells, which gradually normalized over the follow-up period (Figure 1E–F). B cells and NK cells were not affected.

Monocyte HLA-DR density was below reference values and further decreased reaching its nadir on day 7 (Figure

Table. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total No.</th>
<th>Age†</th>
<th>NIHSS‡</th>
<th>Lesion Volume (mm³)§</th>
<th>Localization of Infarction</th>
<th>Thrombolysis</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Nonstroke control subjects*</td>
<td>14</td>
<td>67</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>(50–94)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total patients included</td>
<td>46</td>
<td>70.5</td>
<td>17</td>
<td>172 634</td>
<td>42 MCA</td>
<td>10</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>(41–92)</td>
<td></td>
<td>(2–24)</td>
<td></td>
<td>(441–2 954 304)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected cohort</td>
<td>11</td>
<td>73.4</td>
<td>18</td>
<td>178 875</td>
<td>11 MCA</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>10 pneumonia (1 FUO)</td>
<td></td>
<td>(52–92)</td>
<td>(7–22)</td>
<td>(13 977–2 954 304)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Noninfected cohort</td>
<td>11</td>
<td>63.3</td>
<td>13</td>
<td>111 519</td>
<td>10 MCA</td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>(45–88)</td>
<td></td>
<td>(2–20)</td>
<td></td>
<td>(441–2 551 298)</td>
<td></td>
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</tbody>
</table>

*Nonstroke control subjects were healthy individuals (n=9) or had other neurological diseases (n=5).
†Mean (range).
‡Median (range).
§Median (range).
NA indicates not applicable; MCA, middle cerebral artery; FUO, fever of unknown origin. Three patients additionally had urinary tract infection.

Figure 1. Fluorescence activated cell sorting analysis of nonstroke control subjects (gray bars) and patients with stroke (black bars) on the different days of blood sampling of (a) leukocyte counts, (b) granulocyte counts, (c) percentage of lymphocytes, (d) lymphocyte counts, (e) CD4+ and (f) CD8+ T cell counts, (g) monocyte counts, and (h) HLA-DR expression given as median fluorescence intensity (MFI) on monocytes. *P<0.05; ***P<0.001, mean±SE and the range considered normal in routine laboratory analysis (gray background) are depicted; n (healthy)=14, n (day 0)=33, n (day 1)=41, n (day 7)=39, n (day14)=29.
On day 1, CD4+ T cells ($r = -0.3280$, $P = 0.0444$) and percentage of lymphocytes ($r = -0.4886$, $P = 0.0019$) correlated inversely with National Institutes of Health Stroke Scale on admission but not with infarct volume. Interleukin-6 serum levels were increased on admission and continued to rise throughout the observation period ($P < 0.001$), whereas interleukin-10 did not differ from control subjects.

**Loss of Lymphocytes Was More Pronounced in Patients Who Later Developed Infection**

On admission, no parameter determined in this study differed significantly between the infected and noninfected cohorts (Figure 2). On day 1, however, CD4+ T cell counts recovered more rapidly in patients who remained uninfected compared with patients assigned to the infected cohort. Also on day 1, interleukin-6 concentrations were increased in sera obtained from the infected cohort (Figure 2B, E) Differences in monocyte HLA-DR expression between patient cohorts only became significant on day 7 (Figure 2D).

A regression analysis indicated that CD4+ T cell counts (OR = 6.944; 95% CI = 1.231 to 39.170; $P = 0.028$) and percentage of lymphocytes (OR = 4.598; 95% CI = 1.058 to 19.975; $P = 0.042$) on day 1 were predictors of infection when controlled for the effect of National Institutes of Health Stroke Scale and infarct volume.

**Discussion**

Recent observations in a mouse model have suggested that lymphocytopenia is a cause of infection in the wake of cerebral stroke.3,4 However, attempts to transfer these findings into clinical practice have yielded contradictory results.1,5,6

In this pilot study, we demonstrate a rapid loss of T cells from peripheral blood of patients with stroke. On the day after stroke, absolute numbers of CD4+ T cells differed between the infected and the noninfected cohort due to a lack of recovery in the infected patients.

Our results give additional support to the notion that stroke induces immunosuppression in humans. The loss of CD4+ T-lymphocytes is persistent in patients developing infection suggesting that defects of T helper cell function contribute to immunosuppression in stroke. The findings suggest that low CD4+ T cell counts as well as a low percentage of lymphocytes on day 1 may predispose to infection after cerebral ischemia. If the predictive value of these parameters could be validated in a larger prospective clinical trial, this would provide an early and easily obtainable parameter to identify patients at high risk of subsequent infection.

**Acknowledgments**

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Figure 2. Comparison of noninfected (open circles) and infected (filled circles) patients with stroke for (a) granulocyte counts, (b) CD4+ T cell counts, (c) percentage of lymphocytes, (d) HLA-DR given as median fluorescence intensity (MFI) on monocytes, and (e) serum interleukin-6 levels. nFACS (noninfected): day 0 = 5, day 1 = 11, day 7 = 11, day 14 = 9; nFACS (infected): day 0 = 11, day 1 = 10, day 7 = 10, day 14 = 6; nIL-6 (noninfected): day 0 = 7, day 1 = 9, day 7 = 8, day 14 = 7; nIL-6 (infected): day 0 = 8, day 1 = 9, day 7 = 8, day 14 = 6. Mean ± SE is depicted. *P < 0.05; **P < 0.01; ***P < 0.001.
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Disclosures
None.

References
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