Early immunological defects in comatose patients after acute brain injury

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Object. The aim of this prospective study was to evaluate the phagocytic, humoral, and cellular arms of the immune system in comatose patients shortly after severe brain injury and to compare the findings with those reported earlier in patients in a persistent vegetative state. The study was conducted in intensive care units and immunology laboratories of university-affiliated hospitals in central Israel.

Methods. The study group consisted of 14 men aged 16 to 65 years who were comatose as a result of acute brain injury due to mechanical trauma. All were studied within 72 hours of injury. Brain damage was severe in all cases (Glasgow Coma Scale score < 8). Healthy age- and sex-matched volunteers served as simultaneous controls.

Infections arose in nine (75%) of the 12 patients in whom data were available; the cumulative mortality rate was 38% (five of 13 patients in whom outcome data were available). Every patient exhibited one or more defects in at least one arm of the immune system. Significant deficiencies were noted in neutrophil superoxide release, immunoglobulin (Ig)G, IgG1, IgM, Clq, C2, properdin, alternate C pathway, T cells, T helper cells, T suppressor cells, and natural killer cells. In an earlier series of patients examined by the authors months after the primary insult, these impairments were absent in most of the patients in the vegetative state.

Conclusions. Significant deficiencies of the immune system, particularly the cellular arm, are precipitated by severe brain injury within 72 hours of the event. These impairments probably play a role in the high rate of complicating infections and multiple organ failure. Together with earlier findings, the results of this study indicate that if brain-injured patients survive these hazards, their immune system will eventually recover.

Key Words • head injury • infection • immune system • vegetative state

Severe acute brain trauma has been reported to induce various immunological abnormalities, depending on the location, type, severity, and extent of injury. Several studies so far have dealt with some aspects of the immune status of patients with brain injury, and these reports have implicated mainly the cellular arm, in the form of decreased circulating lymphocytes, depressed T-cell counts, and impaired T-cell activation and proliferation. Only a few, limited studies have been directed at the complement system, the Igs, and the different aspects of the phagocytic arm.

In an earlier study, our team examined the different arms of the immune system in patients in the persistent vegetative state who had severe bacterial infections. The main impairment was found to be in the humoral arm, namely reduction in complement components Clq, C1r, and C4. Also affected were Ig subclasses IgG and IgG1, which substantially influence the phagocytic arm. However, we were unable to determine if these impairments were the result of the primary insult or a complication of prolonged unconsciousness. To resolve this question, we designed the present study to investigate the immunological changes that occur shortly after severe brain injury. We comprehensively evaluated the function and components of the complement system, the Ig subclasses, neutrophil chemotaxis, bactericidal activity, and oxidative burst, and compared these findings with our earlier ones in patients studied months after the primary insult.

Clinical Material and Methods

Patient Population

The study group consisted of 14 men aged 16 to 65 years who sustained an acute severe head injury and who were admitted to the intensive care units of various hospitals in central Israel. The cause of head injury was mechanical trauma in all cases. Patients with concurrent trauma in other parts of their anatomy were excluded.

All patients were clinically evaluated on admission, within 1 hour of the accident, and all scored less than 8 (severe brain damage) on the GCS. There was no histo-

Abbreviations used in this paper: AP50 = alternate C pathway; f-MLP = formyl-methionyleucylphenylalanine; GCS = Glasgow Coma Scale; Ig = immunoglobulin; NK = natural killer; PMA = phorbol myristate acetate; PMNL = polymorphonuclear leukocyte; SE = standard error.
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**TABLE 1**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>GCS Score</th>
<th>Blood Sample†</th>
<th>Infections (days)</th>
<th>Outcome &amp; Time Postinjury</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>5</td>
<td>18</td>
<td>Staphylococcus sp.</td>
<td>conscious, 2,5 mos</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>3</td>
<td>18</td>
<td>coag neg sepsis</td>
<td>died, 3 days</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>6</td>
<td>70</td>
<td>Entrobacter sp.</td>
<td>VS, 28 days</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>3</td>
<td>48</td>
<td>Pseudomonas sp. sep-</td>
<td>conscious, 21 days</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>5</td>
<td>72</td>
<td>Klebiella sp. &amp; Sphynmycocus sp.</td>
<td>conscious, 12 days</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>8</td>
<td>12</td>
<td>rt lobar pneumonia</td>
<td>conscious, 9 days</td>
</tr>
<tr>
<td>7</td>
<td>51</td>
<td>7</td>
<td>72</td>
<td>rt lobar pneumonia</td>
<td>conscious, 12 days</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
<td>6</td>
<td>36</td>
<td>bilat pneumonia</td>
<td>conscious, 13 days</td>
</tr>
<tr>
<td>9</td>
<td>33</td>
<td>3</td>
<td>48</td>
<td>ND</td>
<td>died, 6 mos</td>
</tr>
<tr>
<td>10</td>
<td>39</td>
<td>7</td>
<td>34</td>
<td>Pseudomonas sp. UTI</td>
<td>conscious, 21 days</td>
</tr>
<tr>
<td>11</td>
<td>44</td>
<td>3</td>
<td>26</td>
<td>NIP</td>
<td>died, 2 days</td>
</tr>
<tr>
<td>12</td>
<td>56</td>
<td>4</td>
<td>28</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>54</td>
<td>5</td>
<td>38</td>
<td>NIP</td>
<td>died, 7 days</td>
</tr>
<tr>
<td>14</td>
<td>65</td>
<td>3</td>
<td>68</td>
<td>Pseudomonas sp. sep-</td>
<td>died, 7 days</td>
</tr>
</tbody>
</table>

* Coag neg = coagulase negative; ND = no data available; NIP = no infection proven; UTI = urinary tract infection; VS = vegetative state.
† Data presented in hours postinjury.

**TABLE 2**

<table>
<thead>
<tr>
<th>PMNL Stimulation</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemotaxis (cells/field)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/o f-MLP</td>
<td>112 ± 5</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>w/o f-MLP</td>
<td>49 ± 4</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>net (Δ)</td>
<td>63 ± 5</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>superoxide generation (nmol O₂⁻/10⁶ PMNLs/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f-MLP</td>
<td>1.78 ± 0.22</td>
<td>2.54 ± 0.26</td>
</tr>
<tr>
<td>PMA</td>
<td>2.52 ± 0.23</td>
<td>3.77 ± 0.19</td>
</tr>
<tr>
<td>bactericidal activity (log decrease in cfu)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>autologous serum</td>
<td>0.93 ± 0.11</td>
<td>1.05 ± 0.06</td>
</tr>
<tr>
<td>homologous serum</td>
<td>1.07 ± 0.07</td>
<td>0.90 ± 0.05</td>
</tr>
</tbody>
</table>

* Different numbers of control volunteers were used for the various assays, which were performed at different institutions. Values are expressed as the means ± SE. Abbreviation: cfu = colony-forming units.
† Significant at p < 0.01 level compared with controls.
‡ Significant at p < 0.0005 level compared with controls.

Random migration and chemotaxis were studied in a 48-well chemotaxis chamber (Neuroprobe, Bethesda, MD), as described by Falk, et al.14

The average number of cells in nine fields was counted with the aid of a 20 × objective lens and optical grid and 10 × magnification. Net chemotaxis was calculated by subtracting random migration from chemotactic activity (Table 2). All procedures were performed in duplicate.

The bactericidal activity of PMNLs was expressed as the decrease in the number of viable bacteria after their incubation with PMNLs in the presence of autologous or homologous serum, as described by Clawson and colleagues.13 Each step included three controls, namely, bacteria in phosphate-buffered saline/glucose-albumin, and bacteria in either kind of serum. The number of colony-forming units was counted and their log decrease was calculated and compared with that in controls.

Superoxide production by PMNLs was measured by the reduction of superoxide dismutase–inhibitable ferricytochrome C by the method reported by Weisbart, et al.34 Stimulants with different modes of action were used to define the sites of alteration in signal transduction of superoxide generation: f-MLP was used for receptor-mediated superoxide formation; PMA was used for intracellular protein kinase C–mediated superoxide formation. Superoxide anion release was calculated using the Massey extinction coefficient of 2.1 × 10² M⁻¹ cm⁻¹. Experiments were performed in duplicate, and the results were compared with the findings obtained in basal conditions.

To measure IgG, IgG subclasses, IgM, IgA, IgE, and all complement components and function, venous blood was kept at room temperature for 30 minutes and then centrifuged at 3000 rpm for 3 minutes at 37°C. The serum was separated and kept in aliquots at −70°C. The IgGs were measured using nephelometry; IgE was measured by radioimmunoassay; and IgG subclasses were quantitated by radial immunodiffusion,17 which was also used for the various components of complement.24 Total hemolytic activity of the classic C pathway, CH50, was evaluated using hemolysin-coated sheep erythrocytes,20 and of the AP50

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by using a microtitration assay with rabbit erythrocytes. An average normal value for each complement component was calculated by studying the factors in healthy control volunteers. All values lower than two standard deviations of the mean from normal levels were considered low.

Lymphocyte surface marker analysis was performed using the Q-prep workstation (Coulter, Nyon, Switzerland) and Cyto-stat monoclonal antibodies (Beckman, Nyon, Switzerland).

**Statistical Analysis**

Both the Student t-test and the Mann–Whitney non-parametric test were used for data analysis of all leukocyte functions. The Pearson correlation coefficient was used to assess the statistical correlation among the various parameters of the immune system and the correlation of GCS score, sampling time, and the various immunological parameters.

**Results**

The clinical features, complicating infections, and short-term outcome in each patient are summarized in Table 1. Clinical follow up was feasible in 12 patients, of whom 75% contracted localized or systemic infection within 2 weeks of admission. No significant correlation was found between sampling time and the findings for the immunological parameters; therefore, these data were excluded for the statistical analysis.

In the phagocytic arm, the mean superoxide generation was significantly lower than that in the healthy control volunteers (Table 2). The mean chemotaxis, random migration, and bactericidal activities were within the normal range. Neutrophil f-MLP- and PMA-induced superoxide anion release was reduced in 50% of the patients by one or both pathways (Fig. 1).

In the humoral arm, there was a significant reduction in IgG, IgG1, and IgM (43% of patients) and in some components of the complement system, namely C1q, C2, and properdin (57% of patients) (Table 3). Furthermore, AP50 was significantly reduced, and there was a high correlation between the individual AP50 values and properdin levels (r = 0.8, p < 0.001). We speculated that the low levels of properdin were responsible for the diminished activity of AP50.

The cellular arm was severely impaired in all but three patients (79%). The numbers of circulating T cells, T helper cells, T suppressor cells, and NK cells were significantly reduced (Table 4). Because CD4 and CD8 were decreased to the same extent, the CD4/CD8 ratio remained in the normal range. Also, B-lymphocyte (CD19) levels were within the normal range.

By the time of this analysis, five (38%) of the 13 patients in whom outcome data were available had died, four of them severe brain damage (GCS score ≤ 5) and multiple organ failure within the 1st week after the event. No information could be obtained concerning the fifth death. Comparison of these patients’ immunological status with that of the survivors yielded a significant antimortem impairment in the humoral arm (IgG: 744 ± 203 mg/dl compared with 1043 ± 251 mg/dl in survivors, p = 0.03). The phagocytic and cellular arms were affected to a similar extent. Only in one deceased patient could a bacterial infection be proven (Pseudomonas aeruginosa sepsis and urinary tract infection).
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TABLE 3
Abnormal findings for humoral immunity in 14 comatose patients*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Patients</th>
<th>Controls</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (mg/dl)</td>
<td>936 ± 72‡</td>
<td>1385 ± 76</td>
<td>600–1800</td>
</tr>
<tr>
<td>IgM</td>
<td>484 ± 48‡</td>
<td>825 ± 27</td>
<td>350–1150</td>
</tr>
<tr>
<td>Complement system activity (U/ml)‡</td>
<td>85 ± 14‡</td>
<td>156 ± 25</td>
<td>50–250</td>
</tr>
<tr>
<td>C1q</td>
<td>0.77 ± 0.32‡</td>
<td>1.00 ± 0.09</td>
<td>&lt;0.9</td>
</tr>
<tr>
<td>C2</td>
<td>0.77 ± 0.15**</td>
<td>1.02 ± 0.11</td>
<td>&lt;0.9</td>
</tr>
<tr>
<td>Properdin</td>
<td>0.80 ± 0.14**</td>
<td>1.01 ± 0.11</td>
<td>&lt;0.9</td>
</tr>
<tr>
<td>AP50</td>
<td>126 ± 27††</td>
<td>151 ± 19</td>
<td>120–210</td>
</tr>
</tbody>
</table>

* Values are expressed as the means ± SE. Nine control volunteers were used for Ig testing.
† Significant at p < 0.005 level compared with controls.
‡ Significant at p < 0.001 level compared with controls.
§ Results in this section are expressed as a ratio of the pooled sera of 29 healthy control volunteers.
** Significant at p < 0.02 level compared with controls.
†† Significant at p < 0.01 level compared with controls.

Discussion

Tissue damage, multiple organ failure, and infections are important contributors to the morbidity and mortality associated with acute severe brain injury. In our study, bacterial infections developed within the first 2 weeks of injury in 75% of the patients with acute severe brain trauma. This high rate may well be explained by the immune deficiencies observed in these patients; every patient had one or more defects in at least one arm of the immune system.

Phagocytic Arm

A significant mean decrease in f-MLP- and PMA-induced superoxide generation by neutrophils was noted in 50% of the patients, half of whom got bacterial infections. The absence of a decrease in bactericidal activity indicates that either the deficiency was minor or a bactericidal compensatory mechanism was present. In only two of the patients with a significant decrease in superoxide anion release was there a corresponding decrease in bactericidal activity: one had severe bacterial sepsis but eventually recovered, and the other died of multiple organ failure.

It is well known that a significant decrease in the respiratory burst of phagocytic cells is associated with severe, recurrent, opportunistic bacterial or fungal infections. In our series, the subnormal mean superoxide generation and deficient bactericidal activity of the PMNLs in 50% of the patients correlated with the onset of the postexcitatory phase of PMNL activation. This is consistent with the two-hit model of PMNL activation proposed by Botha, et al., and supported by the earlier finding of Ig deposits in skin biopsy samples obtained in patients with nondrug-induced coma. The deficiencies we observed in several components of the complement system may also have been consumption induced.

Humoral Arm

Immunoglobulins and complement, the major components of the humoral arm of the immune system, mediate opsonization, an essential precondition for phagocytosis and killing of bacteria in PMNLs. In the present study, the defective bactericidal activity of the PMNLs (18% of patients) was correctable in vitro in most cases by the addition of homologous serum. This indicates the presence of a humoral deficiency. Indeed, the observed deficiencies in IgG and IgG, could explain the defective phagocytosis. These findings are consistent with those reported by Moret, et al., in a similar setting. Miller, et al., also reported diminished levels of IgG on the 1st day after head injury. Because the number of B lymphocytes (CD19) was within the normal range, it was probably not the diminished synthesis of the B lymphocytes but rather their sequestration that caused the Ig deficiency—a sort of consumption hypogammaglobulinemia. This notion is supported by the earlier finding of Ig deposits in skin biopsy samples obtained in patients with nondrug-induced coma. The deficiencies we observed in several components of the complement system may also have been consumption induced.

According to our previous study, the total IgG and IgM levels are normal in the vegetative state, as are most of the component levels and functions of the complement system. This situation probably represents recovery of the humoral arm from an earlier stage of deficiency (the vulnerable window). The impaired bactericidal activity of the PMNLs in 27% of the patients who were in the vegetative state were tested during the first 24 hours. Only the patient in whom samples were obtained within 12 hours of the event showed a significant increase in superoxide generation. This finding is in accordance with the hyperactive phase of the two-hit model of neutrophil activation, as proposed by Botha, et al.
state was probably caused by isolated deficiencies of IgG subclasses and some complement components. Because the abnormalities noted in patients who were in the persistent vegetative state were found to be absent within the time frame to which the present study refers, they might be unrelated to the primary insult.

**Cellular Arm**

In the patients in this series, the cellular arm of the immune system was the one most profoundly affected: circulating T cells, T helper cells, T suppressor cells, and NK cells were markedly reduced in number (Table 4). Other investigators have also noted striking deficiencies in the cellular arm as part of the early response to serious brain injury. Some have reported defective activation of the interleukin-2 receptor, anergy (delayed hypersensitivity), and increased levels of tumor necrosis factor-alpha and interleukin-1. Apparently, the cellular arm of the immune system is particularly vulnerable to major trauma. Its compromise probably plays a critical role, by way of multiple organ failure or overwhelming infection, in the worsening course of patients.

**Treatment Options**

In view of the present and previous findings, we assert that PMNLs and immunoproteins are in a state of flux after major trauma in general and brain injury in particular. They could be activated or depressed, under-produced or sequestered, depending on the nature of the insult and the time elapsed since its occurrence. A number of stage-dependent therapeutic strategies have been proposed to help offset the harmful effects of trauma-pre-cipitated immune deficiency. As adjuncts to early surgical and supportive medical management, intravenously administered Ig, fresh-frozen plasma, and certain drugs could be beneficial. Although intravenous Ig administration showed no effect in children with head trauma, some have reported defective activation of the interleukin-2 receptor, anergy (delayed hypersensitivity type), and increased levels of tumor necrosis factor-alpha and interleukin-1. Apparently, the cellular arm of the immune system is particularly vulnerable to major trauma. Its compromise probably plays a critical role, by way of multiple organ failure or overwhelming infection, in the worsening course of patients.

**Conclusions**

Severe brain injury precipitates significant deficiencies of the immune system within 72 hours of occurrence. The cellular arm is the most affected, although phagocytic and humoral deficiencies are also detectable. These impairments could play an important role in the high rate of complicating infections. The deficiencies noted soon after brain injury were not present months after the primary insult in most patients in the vegetative state. This indicates that if brain-injured patients survive the aforementioned hazards, their immune systems will eventually recover.

**Acknowledgment**

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**References**


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