The Relationship of Aging to Endotoxin Shock and to Production of TNF-α

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Background. Aged people are considered prone to gram-negative bacteremia and septic shock. This relationship was tested in murine endotoxin shock.

Methods. Balb/c mice of various ages (1.4-13.4 months) were intraperitoneally injected with lipopolysaccharide (LPS), and rates of survival were observed. The production of TNF-α in vivo induced by LPS was measured.

Results. The survival rates were the smallest in the oldest and youngest groups. Production of TNF-α attained a maximum at 2 h after LPS injection and was smaller in the oldest group; it had a reciprocal relationship to survival rates in each group except the youngest group.

Conclusion. Old and young mice had smaller rates of survival and greater production of TNF-α following endotoxin shock induced by LPS.

THE shock induced by infection with gram-negative bacteria is due to lipopolysaccharide (LPS) from endotoxin of the bacterial cell wall (1). The incidence of gram-negative sepsis is increasing (2,3). Aged patients appear prone to gram-negative bacteremia (4), but it has not been proven experimentally in controlled conditions.

The identification of various mediators or cytokines (5) synthesized and released by macrophages and monocytes (6) helps our understanding of the pathophysiology of gram-negative sepsis. Of these substances, the tumor necrosis factor (TNF) appears to be an important mediator (7-10). The administration of endotoxin to human volunteers results in liberation of free TNF in plasma accompanied by clinical manifestations similar to gram-negative infection (11).

In this investigation, we measured the mortality rates of balb/c mice of various ages following endotoxin shock induced by LPS. The production of TNF-α in sera was also measured. According to the results, the susceptibility to endotoxin shock and production of TNF-α after LPS introduction was correlated with age.

Materials and Methods

Mouse. — Balb/c mice from the same breeders were collected periodically and kept in the same animal room. Some mice in each group were used to test survival rates after LPS-induced endotoxin shock; other mice were used to test the LPS-induced production of TNF-α. For survival tests, the body weights by the end of collection (more than one year) appear in Table 1. The weight increased with age between 1.4 m and 2.6 m and reached a plateau thereafter.

Survival rates. — At the end of collection, mice of each group were intraperitoneally injected with E. coli LPS (0.6 mg/mouse, lot 31H4000, Sigma Chemical Company, St. Louis, MO) in PBS (phosphate buffered saline, containing phosphate .01 M and NaCl .15 M, pH 7.0). Following LPS injection, their survival periods were recorded up to 3 days. If they did not die, they generally survived after that period.

Production and determination of TNF-α. — Mice of each group were similarly injected intraperitoneally with LPS, 0.6 mg per mouse. Before injection and 1, 2, 3, and 4 h after injection, whole blood was collected by eye puncture. Sera were collected to determine TNF-α. TNF-α was assayed in duplicate with an Elisa kit (R & D Systems, Inc., Minneapolis, MN).

Statistical analysis. — The effects of age and body weight on the mouse mortality induced by LPS were analyzed according to the Cox Proportional Hazard Model. The effects of mouse age and period following LPS stimulation in vivo on the production of TNF-α were analyzed by two-way ANOVA with repeated measurement.

Results

Survival period. — The period of survival was recorded at 1, 1.5, 2, and 3 days after injection of LPS. As shown in Figure 1, the survival rate was poor for the young (1.4 m, 2.6 m, 3.3 m) and the old (13.4 m) groups. The oldest group had the poorest survival. Some variation existed in groups before age 4 months; mice were weaned at age 4 weeks. The
Table 1. Age, Number, and Body Weight of Mice in Each Group Used To Study the Survival Rate

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (mo)</th>
<th>n</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13.4</td>
<td>15</td>
<td>29.1 ± 2.0</td>
</tr>
<tr>
<td>B</td>
<td>9.4</td>
<td>5</td>
<td>26.5 ± 1.4</td>
</tr>
<tr>
<td>C</td>
<td>5.5</td>
<td>10</td>
<td>27.6 ± 2.2</td>
</tr>
<tr>
<td>D</td>
<td>4.4</td>
<td>15</td>
<td>28.7 ± 3.3</td>
</tr>
<tr>
<td>E</td>
<td>3.3</td>
<td>15</td>
<td>29.2 ± 1.8</td>
</tr>
<tr>
<td>F</td>
<td>2.6</td>
<td>15</td>
<td>28.3 ± 1.7</td>
</tr>
<tr>
<td>G</td>
<td>1.8</td>
<td>10</td>
<td>25.7 ± 0.9</td>
</tr>
<tr>
<td>H</td>
<td>1.4</td>
<td>15</td>
<td>23.5 ± 1.3</td>
</tr>
</tbody>
</table>

The TNF-α production increased with time after LPS injection, reached maximum at 2 h, and then slowly decreased thereafter (Figure 2).

Analysis by two-way repeated-measurement ANOVA shows that the main effect of age is not related to the production of TNF-α ($p = .115$). Figure 3 shows increased production of TNF-α in the oldest group (group A, 13.4 m) and group E (3.3 m). Both groups had the poorest survival rates. This relationship between TNF-α production and survival rate was inapplicable to the youngest group (group H, 1.4 m), which had a poor survival rate and a small production of TNF-α.

DISCUSSION

This study shows that endotoxin shock induced by LPS has the greatest rate of mortality in aged mice, which is in accord with human statistical evidence that aged patients are prone to gram-negative bacteremia (4).

The age of mice chosen was well-suited for this study, as both youngest and oldest groups showed great rates of mortality, i.e., 80% and 100%, respectively, on the third
day (Figure 1). The LPS dosage used was fixed at 0.6 mg per mouse because we thought that older mice (hence a greater body weight) were not necessarily more tolerant of LPS. The dose 0.6 mg LPS per mouse was chosen according to our previous experience to be about LD₅₀ (the dose lethal to half the mice).

The group E (3.3 m old) had a small rate of survival (6.7%) comparable with that of the oldest group (group A) with 100% mortality as shown in Figure 1, for which there is no satisfactory explanation. Both groups E and A had a small rate of survival and a great production of TNF-α (1950 pg/ml for group E and 2375 pg/ml for group A two hours after LPS injection) (Figures 1 and 3). It seems there is a positive relationship between mortality rates and TNF-α production. This relationship is in accord with human study with meningococemia that showed great concentrations of free TNF in plasma to be associated with morbidity and mortality (12). Antibodies directed against TNF significantly increased the survival of experimental animals in other studies (13–16). Our study indirectly supports the concept that the severity of endotoxin shock is positively related to TNF production. This concept holds for all groups of mice tested except group H, the youngest group.

Group H has both a small rate of survival (20%) and a small production of TNF (595 pg/ml two hours after LPS injection) (Figures 1 and 3). Possibly the dose of LPS used was relatively excessive for this youngest group. Another possibility is that other factors are involved in causing mortality of septic shock. These factors might include cardiopulmonary conditions (17), glucocorticoid potential (18,19), pituitary-adrenal responses (20), sympathetic nerve system (21), and other cytokines such as IL-1 (8,9,22) and IL-6 (23,24).

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REFERENCES


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