Effect of Antimacrophage Serum on Antibody Production and Phagocytosis in Mice

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During the last year four reports have appeared in the literature on the effect of antimacrophage serum (AMS) on phagocytosis and antibody production (1-4). There is general agreement that AMS is cytotoxic to macrophages (1-4) and can decrease the phagocytic activity of peritoneal macrophages (1-4), but some investigators find no immunosuppressive effects after AMS treatment (1, 3), and others (2) report immunosuppression only under certain conditions of antigen administration.

In this report we present evidence that AMS, which decreases the phagocytic activity of mouse peritoneal macrophages, also inhibits primary antibody synthesis. The immunosuppressive effect, however, can only be detected after pretreatment with AMS and low doses of antigen.

Rabbit anti-mouse macrophage serum (AMS) was prepared by injecting New Zealand rabbits three times at bi-weekly intervals, i.v., with $10^8$ or $10^9$ peritoneal cells from C3H/HeJ donor mice. Peritoneal cell donors were injected 7 days previously with 3 ml of thioglycollate medium (5). Peritoneal cells were collected as described before (5). Eighty-six per cent of the thioglycollate-induced peritoneal cells were large macrophages (5). Rabbit serum collected 7 days after the last injection was decomplemented and absorbed with sheep and mouse red blood cells. Control normal rabbit serum (NRS) was similarly treated. The hemagglutinating titers to mouse red blood cells in the initial AMS preparations were high and we found it necessary to absorb at least 4 to 5 times with a packed cell/volume ratio of 1:5. Unabsorbed sera were toxic when injected into mice.

C3H/HeJ mice were injected i.p. with 0.5 ml of $10^9$, $10^8$, or $0.1\times10^8$ sheep red blood cells (SRBC). AMS and NRS were injected i.p. in 0.1-ml volumes. Mouse sera were collected 4 and 11 days after antigen administration by orbital bleeding and the sera from each group of five mice were pooled. Serum antibody titers were determined by hemagglutination before and after treatment of the sera with 2-mercaptoethanol (ME) (5). Titers were recorded as zero if no agglutination took place in the first cup, which had a starting dilution of 1:16. If more than one group

TABLE I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Mice</th>
<th>Phagocytic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MPM with SRBC No. of SRBC/MPM</td>
</tr>
<tr>
<td>AMS</td>
<td>23</td>
<td>8 2</td>
</tr>
<tr>
<td>NRS</td>
<td>23</td>
<td>54 6</td>
</tr>
</tbody>
</table>

* Peritoneal cell (PC) donors injected i.p. with $10^8$ SRBC and 0.1-ml serum. PC collected 1 hr later for determination of phagocytic activity.

b Average values from three separate experiments.

TABLE II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Mice</th>
<th>Log₂ Antibody Titer after Antigen (Ag)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-ME* treatment</td>
</tr>
<tr>
<td>AMS + Ag</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Ag</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>3 days</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>NRS 3 days</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Ag</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

* Treatment within 2-mercaptopoethanol.
TABLE III

Effect of 0.1 ml of antimacrophage serum (AMS) or normal rabbit serum (NRS) injected i.p. 3 days before the antigen on antibody production to 10^7, 10^8 or 10^9 SRBC

<table>
<thead>
<tr>
<th>Serum</th>
<th>SRBC Dose (i.p.)</th>
<th>No. of Mice</th>
<th>Log₂ Antibody Titer after Antigen</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 days</td>
<td>11 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ME treatment</td>
<td>ME treatment</td>
</tr>
<tr>
<td>AMS</td>
<td>10^7</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>NRS</td>
<td>10^7</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>AMS</td>
<td>10^8</td>
<td>20</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
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<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>NRS</td>
<td>10^8</td>
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<td>7</td>
<td>8</td>
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<tr>
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<td>5</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
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<td>5</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

* Treatment with 2-mercaptoethanol.

Table I shows that antimacrophage serum (AMS) reduced the phagocytic activity of mouse peritoneal macrophages. Phagocytic activity was measured by counting, in Wright-stained smears, the number of SRBC-containing macrophages/100 cells as well as the average number of sheep red blood cells (SRBC)/macrophage.

Table II shows that AMS had no effect on serum antibody titers when it was injected simultaneously with the antigen. If, on the other hand, AMS was injected 3 days before 10^8 SRBC, antibody production was decreased both at 4 and 11 days after antigen was administered.

Table III shows that the immunosuppressive effect of AMS was antigen-dependent. AMS had no effect on antibody production to 10^9 SRBC, lowered the production of antibody to 10^8 SRBC, and practically eliminated the production of antibody to 10^7 SRBC. The data also indicate that the antibody produced 11 days after injecting 10^7 SRBC was completely ME-sensitive in animals treated with AMS.

The results indicate that AMS, which reduces the phagocytic activity of mouse peritoneal macrophages, also has an immunosuppressive effect. The immunosuppressive effect, however, can best be detected after pretreatment with AMS and low doses of antigen. We are currently investigating whether there is any correlation between the suppression of phagocytic activity and the decreased antibody titers. Experiments to test whether AMS affects both macrophages and lymphocytes or macrophages alone are in progress.

REFERENCES

2. J. Paniel and P. Cayeux, Immunology, 14: 769, 1968.