Adjuvant Action of Bacterial Endotoxin and Colchicine on Antibody Formation in the Hamster

KATHARINE MERRITT

Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03755

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Endotoxin was demonstrated not to act as an adjuvant in the primary antibody response in the Syrian hamster. Minimal adjuvant action was demonstrable in antibody formation to a second injection of bovine gamma globulin. The immunological status of the hamster towards the endotoxin did not alter this weak adjuvant action. It was confirmed that the antimitotic drug colchicine will act as an adjuvant in the primary antibody response to sheep red blood cells but not to bovine gamma globulin or endotoxin in the Syrian hamster.

Bacterial endotoxin has been shown to be an adjuvant to antibody formation in several animal species. The exact mechanism by which endotoxin exerts this adjuvant action remains undefined, but evidence supports the hypothesis that endotoxin damages lymphoid cells and releases nucleic acids (4) which will then enhance antibody formation (7).

In previous experiments, bacterial endotoxin was found incapable of causing a generalized Shwartzman reaction (GSR) in the Syrian hamster under a variety of conditions, and it was not immunogenic after a single injection into these animals (6). Endotoxin was, however, toxic to hamsters as evidenced by huddling, conjunctivitis, and fetal death. Since there was no detectable natural antibody and no detectable primary antibody response to endotoxin, it was felt that the hamster provided a system for investigating the role of immunity to endotoxin on its ability to act as an adjuvant. In addition, since colchicine causes a disease in pregnant Syrian hamsters indistinguishable from the GSR (2) and has been shown to be a weak but definite adjuvant to antibody formation in the hamster (3), experiments were undertaken to study its role as an adjuvant to antibody formation in the Syrian hamster in this laboratory.

MATERIALS AND METHODS

Animals. Syrian hamsters, Mesocricita aurataus Waterhouse (1939), were purchased from the Lakeview Hamster Colony, Newfield, N.J., and were maintained on Purina Lab Chow and water ad lib. The hamsters were 10 to 12 weeks of age when used.

Animals were injected intraperitoneally or intravenously by way of a lateral lingual vein.

Endotoxin. Bacterial lipopolysaccharides prepared by the Boivin method from Escherichia coli 0127:B8 (control 463366) and by phenol extraction from Salmonella typhosa 0-901 (control 473454) were purchased from Difco. These lipopolysaccharides were dissolved in 0.15 M NaCl and administered in the dose and route indicated. These preparations were capable of producing the GSR in rabbits and of enhancing antibody formation to bovine gamma globulin in Balb mice. The endotoxins were effective antigens in mice.

Colchicine. Colchicine USP (Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in 0.15 M NaCl and administered intraperitoneally in a dose of 3 or 10 mg per hamster.

Sheep red blood cells (SRBC), collected in Alsever’s solution, were purchased from Scott Laboratories, Fiskeville, R.I. The cells were washed and resuspended to 2×10^7 in 0.15 M NaCl. Hamsters were injected intraperitoneally or intravenously with 0.15 ml of the cell suspension.

Antibody determinations. Hamsters under pentobarbital anesthesia were bled by cardiac puncture through the intact chest wall. Antibody to SRBC was determined by direct hemagglutination of the SRBC suspension, whereas antibody to BGG was detected by the passive hemagglutination technique of Boyden (1). Antibody titers are recorded as the reciprocal of the highest dilution of serum in a twofold serial dilution that caused agglutination of the cells. The antibody response of hamsters within a single experi-
mental group is uniform, differing by a twofold serial dilution which is the error of the test system.

RESULTS

Effect of endotoxin on the primary immune response. Hamsters were injected intraperitoneally with 0.5 ml of the SRBC suspension and doses of endotoxin varying from 10 to 300 μg. As can be seen from the results of one experiment representative of the five experiments with six hamsters in each (Table 1), there was no adjuvant effect of endotoxin as determined by a shortened induction period or an increase in peak titer. Similar results were obtained with 5 and 100 μg of endotoxin.

Inasmuch as most investigators have employed soluble protein antigens to measure the adjuvant action of bacterial endotoxin, experiments were undertaken in the hamster with BGG as the antigen. Hamsters were injected with endotoxin at various times to determine if endotoxin given before, with, or after BGG (3 or 10 mg) would stimulate antibody formation. The protocol and results depicted in Table 2 demonstrate that endotoxin given at various times in relation to antigen did not stimulate the production of a detectable primary antibody response to BGG in these hamsters. Such results were obtained in three experiments with three hamsters per group.

The effect of immunity to endotoxin on its ability to act as an adjuvant was then investigated. Neither hamsters which had been immunized previously with endotoxin and would now respond with a rapid production of antibody nor hamsters with circulating antibody to endotoxin produced a detectable primary response to BGG given with endotoxin. Antibody titers on days 7, 14, and 21 were 0.

Effect of endotoxin on the secondary immune response. Although Syrian hamsters do not form detectable antibody to a single injection of BGG, they do form high titers of antibody after a second injection of the antigen. Accordingly, experiments were undertaken to test the effect of endotoxin given (i) with the antigen in the first injection, (ii) with the antigen in the second injection, and (iii) with both the first and second injections of antigen. The data recorded in Table 3 indicate that endotoxin acts as an adjuvant to the second-

<table>
<thead>
<tr>
<th>Determination</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
<th>Day 8</th>
<th>Day 12</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRBC</td>
<td>&lt;10</td>
<td>80</td>
<td>1,280</td>
<td>1,280</td>
<td>640</td>
<td>320</td>
</tr>
<tr>
<td>SRBC + 50 μg of ET</td>
<td>&lt;10</td>
<td>160</td>
<td>2,560</td>
<td>2,560</td>
<td>640</td>
<td>320</td>
</tr>
</tbody>
</table>

Table 1. Lack of adjuvant action of endotoxin (ET) on antibody formation to sheep red blood cells (SRBC) in the Syrian hamster

<table>
<thead>
<tr>
<th>Time of ET administration (50 μg)</th>
<th>Antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>At 5 days before BGG</td>
<td>2</td>
</tr>
<tr>
<td>At 2 days before BGG</td>
<td>0</td>
</tr>
<tr>
<td>At 1 day before BGG</td>
<td>0</td>
</tr>
<tr>
<td>At 1 hr before BGG</td>
<td>2</td>
</tr>
<tr>
<td>With BGG</td>
<td>2</td>
</tr>
<tr>
<td>At 1 day after BGG</td>
<td>0</td>
</tr>
</tbody>
</table>

* In relationship to antigen administration.

Table 2. Effect of time of administration of endotoxin (ET) on antibody formation to 3 mg of bovine gamma globulin (BGG) in the Syrian hamster

<table>
<thead>
<tr>
<th>Time of ET administration (50 μg)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>None</td>
<td>80</td>
</tr>
<tr>
<td>Given with the first injection of BGG</td>
<td>640</td>
</tr>
<tr>
<td>Given with the second injection of BGG</td>
<td>10</td>
</tr>
<tr>
<td>Given with both injections of BGG</td>
<td>640</td>
</tr>
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Table 3. Adjuvant action of endotoxin (ET) on the secondary immune response to bovine gamma globulin (BGG) in the Syrian hamster

<table>
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</tr>
<tr>
<td>At 5 days before BGG</td>
<td>2</td>
</tr>
<tr>
<td>At 2 days before BGG</td>
<td>0</td>
</tr>
<tr>
<td>At 1 day before BGG</td>
<td>0</td>
</tr>
<tr>
<td>At 1 hr before BGG</td>
<td>2</td>
</tr>
<tr>
<td>With BGG</td>
<td>2</td>
</tr>
<tr>
<td>At 1 day after BGG</td>
<td>0</td>
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</tbody>
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<tr>
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</tr>
<tr>
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<td>10</td>
</tr>
<tr>
<td>Given with both injections of BGG</td>
<td>640</td>
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<tr>
<td>Given with the first injection of BGG</td>
<td>640</td>
</tr>
<tr>
<td>Given with the second injection of BGG</td>
<td>10</td>
</tr>
<tr>
<td>Given with both injections of BGG</td>
<td>640</td>
</tr>
</tbody>
</table>
TABLE 4. Adjuvant action of endotoxin (ET) on the secondary antibody response to bovine gamma globulin (BGG) in Syrian hamsters previously hyperimmunized to the endotoxin

<table>
<thead>
<tr>
<th>Time of ET administration (50 μg)</th>
<th>Antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>None</td>
<td>80</td>
</tr>
<tr>
<td>Given with the first injection of BGG</td>
<td>320</td>
</tr>
<tr>
<td>Given with the second injection of BGG</td>
<td>160</td>
</tr>
<tr>
<td>Given with both injections of BGG</td>
<td>320</td>
</tr>
</tbody>
</table>

TABLE 5. Enhancement of antibody formation to sheep red blood cells by colchicine in the Syrian hamster

<table>
<thead>
<tr>
<th>Dose of colchicinea</th>
<th>Antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>16</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>32</td>
</tr>
<tr>
<td>300 mg/kg</td>
<td>32</td>
</tr>
</tbody>
</table>

a Given with the antigen.

vant action was similar to that in the previous report, but a higher dose (100 mg/kg instead of 25 mg/kg) was needed in this study. The administration of colchicine in various doses with endotoxin did not stimulate the hamster to produce a primary response against these antigens. Similar results were obtained in each of four experiments.

DISCUSSION

Endotoxin causes a variety of responses in experimental animals. The responses most common and most studied are lethality, fever, and shock. However, in addition to these toxic effects, endotoxin has been shown to enhance antibody formation to unrelated antigens and to increase nonspecific resistance to a variety of infectious agents. The mechanism by which endotoxin exerts these effects remains undefined. Investigators have attempted to relate the toxicity of endotoxin to the reaction of the endotoxin with antibody in the host. However, the reports by Kim and Watson (5, 11) on the lethality of endotoxin in immunoglobulin-free piglets indicate that endotoxin is intrinsically toxic without acting through an antigen-antibody complex.

It was hoped that another model system could be developed in the Syrian hamster for studying the effects of endotoxin. The hamster, in contrast to the B10.LP and Balb/c mice used in this laboratory, did not produce a detectable antibody response to a single injection of endotoxin. Attempts to produce the GSR or a pyrogenic response under a variety of conditions were unsuccessful, suggesting that it is a mechanism other than an antigen-antibody complex or sensitized cells that produces the reactions (6). It was hoped that attempts to enhance antibody formation in the hamster with endotoxin would be successful and indicate that the toxic effects of endotoxin and the enhancing effects functioned through different mechanisms. It must be concluded from the data obtained that the Syrian hamster is quite refractory to both the toxic and beneficial effects of endotoxin. The rat and the guinea pig have also been shown to be quite refractory to the effects of endotoxin except that endotoxin will act as a good adjuvant in these species (8–10). Thus, the Syrian hamster should serve as a model for comparison with other animals when studying the toxic nature of bacterial endotoxin or when studying antibody formation. It is of interest that the antimitotic agent colchicine can mimic in the hamster the effects of endotoxin in other species. In preliminary studies on the effect of endotoxin on the lymphoid tissues of hamsters, it appears that there was little alteration of size or cell number. Destruction of lymphoid cells was not observed in the spleen, lymph nodes, or Peyer’s patches after endotoxin treatment, nor was there a marked increase in cellularity in organs examined as long as 9 days after endotoxin administration. Studies on reticuloendothelial activity in hamsters treated with endotoxin and in vitro studies on phagocytosis have not yet been undertaken. The hamster provides an interesting experimental animal for studies on the role of the reticuloendothelial system in the immune response.

LITERATURE CITED

6. Merritt, K., and M. Galton. 1967. Failure of bacterial endo-
toxin to produce the generalized Shwartzman reaction in the Syrian hamster. J. Bacteriol. 94:590–596.


