Induction of Resistance to Ascites Tumor in Mice with MFS-180 Cells Treated with Glutaraldehyde, Lipopolysaccharide, and Concanavalin A

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ABSTRACT

Immunization with MFS-180 vaccine prepared in the presence of glutaraldehyde (0.05%), lipopolysaccharide (LPS) (100 \( \mu \)g/ml), and concanavalin A (Con A) (200 \( \mu \)g/ml) could protect Swiss mice against a subsequent challenge by \( 1 \times 10^6 \) MFS-180 cells. The sequence of attachment of LPS and Con A to glutaraldehyde-treated cells was found to determine the efficacy of the vaccine. LPS coupled with glutaraldehyde-treated cells before Con A could effect 100% survival, while LPS attached after Con A treatment to glutaraldehyde-treated cells showed only 60% survival. The protection was specific for syngeneic cells.

INTRODUCTION

Neoplastic cells in general possess weak antigenicity; therefore, efforts are being made to modify their antigenicity by various chemical and physical methods (1, 3). Tumor cells treated with Con A\(^1\) resulted in enhanced immunological resistance (7). Recently, Kataoka et al. (4, 6) obtained highly immunogenic L1210 leukemic cells by treating them with glutaraldehyde and Con A which could protect the animals against leukemia. Nevertheless, Con A-tumor cell vaccine was not sufficiently effective in immunotherapy because it could not offer complete protection, although its potency was clearly demonstrated (5). Bacterial LPS have been shown to be B-cell mitogen as well as macrophage activator (2, 8). We have examined the effect of LPS on the immunogenic properties of MFS-180 cells pretreated with glutaraldehyde and Con A. It was observed that combining LPS first to glutaraldehyde-treated cells and then with Con A was most effective in protecting mice against the challenge with ascites cells.

MATERIALS AND METHODS

Animals. Male Swiss mice used were originally obtained from The Jackson Laboratory, Bar Harbor, Maine, and were maintained in our animal house by brother-sister mating. All animals were used at 6 to 8 weeks of age and were given stock diet and water ad libitum.

Tumor Cells. A transplantable sarcoma which could be grown either as ascites or as solid tumor was obtained from Dr. S. Sato, National Cancer Institute, Tokyo, Japan. Tumor was maintained by i.p. injection of ascitic fluid into mice. A suspension of MFS-180 cells was prepared by drawing ascitic fluid from the peritoneal cavity and washing it 3 times with Eagle’s minimum essential medium. Mouse fibrosarcoma B (solid tumor) was obtained from Cancer Research Institute, Bombay, India. Cell suspension was prepared by trypsinization of the tumor.

Chemicals. Con A and glutaraldehyde were purchased from Sigma Chemical Co., St. Louis, Mo. LPS (Re 595) was obtained from Dr. Chris Galanos, Max-Planck-Institut fur Immunologie, Freiburg, W. Germany.

Preparation of Vaccine Cells. The general procedure followed for preparation of vaccine of MFS-180 cells was similar to that of Kataoka et al. (6). MFS-180 cells were washed 3 times with ice-cold 0.1 M PBS (pH 7.2) and suspended in the same buffer to obtain \( 2 \times 10^7 \) cells/ml. This was then treated with glutaraldehyde (0.05%) for 1 hr at 0\( ^\circ \) and washed 3 times with ice-cold PBS. These cells were then treated with either Con A (200 \( \mu \)g/ml) or LPS (100 \( \mu \)g/ml) for 1 hr on ice and washed with PBS. To Con A-treated cells, LPS (100 \( \mu \)g/ml) was added; to LPS-treated cells, Con A (200 \( \mu \)g/ml) was added. The cells were incubated on ice for 1 hr and then washed 3 times with ice-cold PBS. Thus, various treatments given to MFS-180 cells were (a) control (PBS only); (b) glutaraldehyde; (c) glutaraldehyde plus Con A; (d) glutaraldehyde plus LPS; (e) glutaraldehyde plus Con A plus LPS; (f) glutaraldehyde plus LPS plus Con A.

Optimization of LPS Concentration. Various concentrations of LPS (0 to 150 \( \mu \)g/ml) were used in the preparation of vaccine as described above.

Immunization. MFS-180 cells treated as described above were suspended in PBS (2 \( \times \) 10\(^7\) cells/ml), and 0.5 ml suspension was injected i.p. into mice. A booster dose followed after 1 week. These mice were then challenged with MFS-180 cells (1 \( \times \) 10\(^6\) cells/mouse) 15 days after the booster dose was administered, and their survival was recorded. Those immunized mice that survived the first challenge for 1 month were rechallenged with \( 1 \times 10^6 \) MFS-180 cells. The mice which survived the second challenge for another month were counted as survivors. The mean survival time of the mice which died within 1 month of the first challenge was recorded.

RESULTS

Immunization with Glutaraldehyde- and Con A- or LPS-treated Cells. Different groups of mice were immunized with glutaraldehyde-treated cells, glutaraldehyde-plus-Con A-treated cells, or glutaraldehyde-plus-LPS-treated cells. The control group received PBS only. When these animals were challenged with \( 1 \times 10^6 \) MFS-180 cells, it was observed (Table 1) that control group as well as mice immunized with glutaraldehyde-treated cells did not offer protection to the challenge with ascites cells \( (1 \times 10^6 \) cells/mouse) and the mean survival times were 20.3 and 21.5 days, respectively. In the case of mice immunized with glutaraldehyde- Con A-treated cells or glutaraldehyde- and LPS-treated cells, the number of survivors

\(^{1}\) The abbreviations used are: Con A, concanavalin A; LPS, lipopolysaccharides; PBS, phosphate-buffered saline.

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2922 CANCER RESEARCH VOL. 41

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Induction of Resistance to Ascites Tumor in Mice

Optimization of LPS Concentration for Vaccine Preparation. Optimization experiments with regard to LPS concentration in the preparation of vaccine were conducted, and the results are shown in Table 2. The number of mice that survived after 1 month of challenge with ascites cells increased with increasing concentrations of LPS up to 100 μg/ml, and then the number of survivors decreased. Also, the sequence of LPS and Con A coupling was found to be of significance in vaccine preparation. When glutaraldehyde-treated cells were first treated with Con A and then with LPS, 6 of 10 mice survived. However, treatment of glutaraldehyde-treated cells first with LPS and then with Con A gave 100% protection; i.e. 10 of 10 mice survived.

Immunization of Mice with Glutaraldehyde-, Con A- and LPS-treated Ascites Cells. Three groups of mice were used. One group served as control and received PBS only. The other group of mice was given cells treated with glutaraldehyde, Con A, and LPS, while the third group was given injections of glutaraldehyde-treated cells treated with LPS and then with Con A. As shown in Table 3, all the animals survived in the third group where LPS was coupled before Con A to the glutaraldehyde-treated cells, while in the second group 22 of 30 mice survived after 1 month of challenge. These survivors were rechallenged with 1 × 10⁶ ascites cells per ml and were found to survive the second challenge.

Specificity of Resistance Induced by Immunogenic Cells. For examination of specificity of resistance induced by vaccine, immunized mice were challenged with MFS-8. It was observed that these mice showed no resistance to MFS-8 cells and developed solid tumor, while the immunized mice survived challenge of syngeneic MFS-180 cells.

**DISCUSSION**

The above results demonstrate that administration of ascites cells treated with glutaraldehyde, LPS, and Con A protected mice from the subsequent inoculation of live ascites cells. These mice survived even after a second challenge with MFS-180 cells. Kataoka (5) could obtain some protection in mice against the challenge of syngeneic tumor by coupling glutaraldehyde-treated cells with Con A. However, the protection was not complete, although the majority of mice did develop immunological response. The binding of LPS, which is a B-cell mitogen as well as macrophage activator, to glutaraldehyde- and Con A-treated cells could bring about complete protection in mice. Our results further indicate that LPS and Con A individually were not effective. Also, the sequence of binding of LPS and Con A to glutaraldehyde-treated cells could determine the efficacy for rendering the cells immunogenic. As seen in Table 3, binding of LPS to glutaraldehyde-treated cells followed by Con A was was most effective, while coupling of Con A to glutaraldehyde-treated cells first followed by LPS was not as effective in protecting the mice.

Immunogenic cells were washed repeatedly before inoculation, making it unlikely that free unbound LPS or Con A contaminating the inoculum was responsible for the induction of resistance. Thus, cell-bound LPS and Con A are associated with the protection of mice. It has been suggested that blastogenesis of glutaraldehyde-Con A vaccine would be associated with the induction of immune resistance (5). Bacterial LPS has been reported to enhance production of antibody-forming cells to a synthetic antigen when both of them are located on the same but not on separate liposome carriers (9). Therefore, it seems that LPS and Con A when bound to glutaraldehyde-treated cells may enhance the formation of antibody cells against tumor antigen and also produce immunoblasts that could react specifically with tumor cells.

### Table 1
**Immunization with glutaraldehyde-Con A- or glutaraldehyde-LPS-treated cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of survivors/total no. of mice used</th>
<th>Survival time (days) of mice died within 1 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/30</td>
<td>20.3 ± 2.1b</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>0/30</td>
<td>21.5 ± 3.31</td>
</tr>
<tr>
<td>Glutaraldehyde + Con A</td>
<td>12/30</td>
<td>25.0 ± 4.1</td>
</tr>
<tr>
<td>Glutaraldehyde + LPS</td>
<td>8/30</td>
<td>25.4 ± 3.7</td>
</tr>
</tbody>
</table>

* Control group received PBS only prior to challenge with MFS-180 cells.  
 b Mean ± S.D.

### Table 2
**Determination of optimum concentration of LPS for vaccine preparation**

<table>
<thead>
<tr>
<th>Sequence of treatment</th>
<th>No. of survivors/total no. of mice used</th>
<th>Survival time (days) of mice died during 1 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no treatment)</td>
<td>0/10</td>
<td>20.3 ± 2.1a</td>
</tr>
<tr>
<td>Con A</td>
<td>3/10</td>
<td>25 ± 4.1</td>
</tr>
<tr>
<td>Con A LPS (25 μg)</td>
<td>3/10</td>
<td>25.5 ± 3.6</td>
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<tr>
<td>Con A LPS (50 μg)</td>
<td>4/10</td>
<td>28.5 ± 2.2</td>
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<tr>
<td>Con A LPS (100 μg)</td>
<td>6/10</td>
<td>27.5 ± 1.0</td>
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<tr>
<td>Con A LPS (150 μg)</td>
<td>2/10</td>
<td>23.5 ± 3.1</td>
</tr>
<tr>
<td>LPS (25 μg)</td>
<td>6/10</td>
<td>25.0 ± 2.5</td>
</tr>
<tr>
<td>LPS (50 μg)</td>
<td>8/10</td>
<td>24.0 ± 1.8</td>
</tr>
<tr>
<td>LPS (100 μg)</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>LPS (150 μg)</td>
<td>5/10</td>
<td>24.0 ± 0.5</td>
</tr>
<tr>
<td>LPS (25 μg)</td>
<td>0/10</td>
<td>25.0 ± 2.1</td>
</tr>
<tr>
<td>LPS (150 μg)</td>
<td>4/10</td>
<td>22.5 ± 1.7</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
REFERENCES


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