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TECHNICAL MANUSCRIPT 153

ANTIGENICITY OF IRRADIATED PASTEURELLA TULARENSIS VACCINES IN MICE

AUGUST 1964

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ABSTRACT

Viable attenuated tularemia vaccines have been shown to be effective in immunizing mice and other animals against challenge with highly virulent Pasteurella tularensis. Preparations made nonviable by the action of heat or chemicals, however, induce little or no resistance to challenge with even small numbers of virulent organisms.

In these studies, vaccines rendered nonviable by the action of ionizing radiation produced levels of immunity such that 20 to 30 per cent of immunized mice survived intraperitoneal challenge with moderate doses of the highly virulent SCHU S4 strain of P. tularensis. Both x- and electron beam-irradiation were effective in preparing the vaccine, which contained from $1.4 \times 10^8$ to $4.0 \times 10^9$ nonviable organisms per milliliter.
Nonviable tularemia vaccines generally have proved to be ineffective for protecting laboratory animals against challenge with a highly virulent strain. This ineffectiveness may be caused by heat or chemicals used to render the organisms nonviable. It seemed reasonable to suppose that antigenicity would be retained to a greater extent if the organisms were killed by irradiation rather than by chemicals or heat. This supposition is based on the hypothesis that under conditions that inhibit the indirect effects of irradiation, damage occurs primarily in the genetic material of the cell, and to a lesser extent to other structures of the cell.

Precedents for the effectiveness of irradiated vaccines may be found in the reports of Donaldson and Mitchell, of Polley, and of Carpenter and his associates. The latter authors have demonstrated that irradiated tubercle bacilli were as effective as BCG vaccine in protecting mice against infection with this organism.

In the present study the highly virulent SCHU S4 strain of Pasteurella tularensis was used for vaccine preparation and challenge. In some of the initial experiments an attenuated strain, designated LVS, was also used for vaccine preparation. The organisms were grown at 37°C for 24 hours on the surface of an agar medium containing peptone, yeast extract, orotic acid, spermidine phosphate, histidine, cysteine hydrochloride and glucose. Cells were washed from the agar with 0.1 per cent gelatin-saline; the resulting suspensions contained in the order of $10^{10}$ viable organisms per milliliter as determined by plate counts.

Two types of ionizing radiation were used to render bacterial suspensions nonviable: (a) x-rays produced by a 1 million electron volt Maxitron machine, and (b) an electron beam generated by a 3 million electron volt Van de Graaff accelerator. Because high doses of irradiation are necessary to sterilize these suspensions, beta-mercaptoethylamine, also referred to as cysteamine, was added to the suspensions at a final concentration of 0.02 M prior to irradiation to minimize the indirect effects of irradiation, that is, to scavenge free radicals produced upon irradiation of water. Following irradiation, the suspensions were tested for sterility, diluted tenfold in gelatin-saline, and injected intraperitoneally in 1.0 milliliter amounts into 18- to 20-gram white mice. The mice were challenged intraperitoneally two weeks later with virulent SCHU S4 organisms and observed for 21 days.

Table I presents the results of an initial x-ray experiment. The first two lines of this table point out that if the dose of irradiation is insufficient to sterilize the suspension, the surviving viable attenuated organisms can immunize mice. The mouse responses listed in the last column are results of a 1:10 dilution of the irradiated preparation. The second line

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*In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.*
### TABLE I. RELATIONSHIPS AMONG CELL CONCENTRATION, IRRADIATION DOSE, AND IMMUNIZING CAPACITY

<table>
<thead>
<tr>
<th>Immunizing Strain</th>
<th>X-Ray Dose (r)</th>
<th>Viable Cells/ml Before Irradiation</th>
<th>Viable Cells/ml After Irradiation</th>
<th>Mouse Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVS</td>
<td>20,000</td>
<td>1.03 x 10^6</td>
<td>790</td>
<td>Immunized</td>
</tr>
<tr>
<td>LVS</td>
<td>30,000</td>
<td>1.03 x 10^6</td>
<td>0</td>
<td>Not Immunized</td>
</tr>
<tr>
<td>SCHU S4</td>
<td>30,000</td>
<td>4.0 x 10^6</td>
<td>320</td>
<td>Death</td>
</tr>
<tr>
<td>SCHU S4</td>
<td>40,000</td>
<td>4.0 x 10^6</td>
<td>0</td>
<td>Not Immunized</td>
</tr>
<tr>
<td>SCHU S4</td>
<td>300,000</td>
<td>1.67 x 10^10</td>
<td>160</td>
<td>Death</td>
</tr>
<tr>
<td>SCHU S4</td>
<td>600,000</td>
<td>1.67 x 10^10</td>
<td>0</td>
<td>Immunized</td>
</tr>
</tbody>
</table>

Indicates that 10^5 killed organisms will not immunize mice. The interpretation of "immunized" was based on 80 per cent of the animals in the first group surviving challenge of approximately 100 LD_{50}; the interpretation of "not immunized" was based on survival of only 10 per cent of the group.

The second two lines point out that 32 viable SCHU S4 organisms killed all of the animals during the immunization period, whereas 4 x 10^5 killed organisms were ineffective in inducing immunity; all animals succumbed to challenge in this fourth group.

In the next to the last line 16 viable virulent organisms killed 80 per cent of the mice prior to challenge. The last line shows that approximately 10^9 irradiated SCHU S4 organisms induced "immunity" -- 50 per cent of the mice survived challenge. The results of this initial work indicated that ionizing radiation was applicable to this system.

Table II presents the results of challenge of mice immunized with x-irradiated SCHU S4 Pasteurella tularensis. The concentration of organisms ranged from 3 x 10^8 to 4 x 10^9 organisms per milliliter; the dose of irradiation was one million roentgens. The figures represent the combined results of seven replicate vaccine preparations. The per cent mortality of immunized mice, given in the center column is the response to challenge with approximately 200 intraperitoneal LD_{50}. The figures in parentheses refer to the range of individual results.
TABLE II. IMMUNOGENICITY OF X-IRRADIATED VACCINE IN MICE

<table>
<thead>
<tr>
<th></th>
<th>Mortality Ratio</th>
<th>Per Cent Mortality</th>
<th>Average Day of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-Irradiated Vaccine</td>
<td>71/89</td>
<td>80 (50 - 95)</td>
<td>11.8 (9.9 - 15.2)</td>
</tr>
<tr>
<td>None</td>
<td>68/68</td>
<td>100</td>
<td>4.4 (4.0 - 4.7)</td>
</tr>
</tbody>
</table>

Preparations of phenol-killed Pasteurella tularensis, which contained $7.5 \times 10^8$ organisms per milliliter, were used to immunize mice, but failed to result in any survivors following challenge with moderate doses of SCHU S4 organisms. The average day of death of this group of mice was about 7.4 days.

Vaccines have also been prepared using an electron beam to render organisms nonviable.

Table III points out the similar results of x- and electron beam-irradiation on the efficacy of vaccine preparations. Five electron beam vaccines were prepared in a manner similar to the x-ray preparations. The vaccines contained from approximately $10^8$ to $10^9$ organisms per milliliter and were irradiated with 1.6 million rad. Groups of mice were immunized and challenged two weeks later with from 100 to 200 LD50 of SCHU S4 organisms. The comparative results presented in Table III demonstrate that electron beam-irradiation is at least as good as x-irradiation for preparing vaccines.

An important consideration in these studies is the assurance of complete sterility of irradiated suspensions because viable attenuated organisms are capable of inducing an appreciable degree of immunity. Proof of nonviability of irradiated vaccines was based on the following observations: (a) no colonies developed when irradiated suspensions were plated on solid medium, (b) viable Pasteurella tularensis could not be isolated from the lung, liver, or spleen of mice sacrificed on the first, third, sixth, or tenth day following immunization, (c) administration of streptomycin to mice starting one day before and ending six days after a single immunizing injection did not interfere with the development of immunity. Such streptomycin
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</thead>
<tbody>
<tr>
<td>X-Irradiated Vaccine</td>
<td>71/89 (50 - 95)</td>
<td>11.8 (9.9 - 15.2)</td>
</tr>
<tr>
<td>Electron Beam-Irradiated Vaccine</td>
<td>164/236 (50 - 100)</td>
<td>12.8 (7.6 - 15.6)</td>
</tr>
<tr>
<td>None</td>
<td>117/117 (3.9 - 5.4)</td>
<td></td>
</tr>
</tbody>
</table>

treatment inhibits the development of immunity that normally follows administration of less than $10^4$ viable attenuated organisms.

Irradiated vaccines retained immunogenicity after seven days at $5^\circ$C, but had lost this immunogenicity by the fiftieth day of storage.
LITERATURE CITED

