SUBCUTANEOUS IMMUNIZATION OF GUINEA PIGS WITH DICTYOCOALUS VIVIPARUS LARVAE ATTENUATED BY X-RAY IRRADIATION

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Abstract. Groups of guinea pigs were immunized s/c with 2 doses of 5000 infective Dictyocaulus viviparus larvae attenuated by exposure to 20, 30, 40 and 50 kR. Twelve days after the second inoculation they were challenged orally with 5000 infective Dictyocaulus viviparus larvae. Protective immunity was assessed on the basis of larval recovery from guinea pig lungs 7 days after challenge. All groups that received irradiated larvae showed approximately the same immunizing effect except for the 40 kR group where the average lung burden was influenced by an extremely high lung burden in one animal. In the control group inoculated s/c with unirradiated larvae the immunizing effect was found to be markedly higher.

Radiation-attenuated vaccines against dictyocaulosis in cattle and sheep produced to date are generally administered orally in the form of aqueous suspension. In other words, the vaccines are given by using the natural route of infection. Some experiments, however, have also been designed to induce immunity with living unirradiated lungworm larvae administered by other routes. Such experiments are based on recent knowledge regarding active immunization against helminth infections requiring the presence of living parasites at a certain developmental stage in the host’s organism.

Davtyan and Shults (1956) who administered large quantities of Dictyocaulus filaria larvae to sheep using intraperitoneal and intramuscular routes claimed a certain degree of success but made no mention of differences in comparison with controls. Cornwall (1962) produced immunity to Dictyocaulus viviparus in calves by i/p administration of fourth-stage Dictyocaulus viviparus larvae recovered from the calf lung. Wade and Swanson (1958) produced successful subcutaneous infection of calves with large doses of first- and third-stage Dictyocaulus viviparus larvae. These writers, however, were not concerned with immune response. Production of immunity to Dictyocaulus infection by oral administration of lungworm larvae in guinea pigs serving for model experiments was reported by a number of writers (Wade et al. 1960, Poynter et al. 1960).

In an earlier study performed in this laboratory it was found that subcutaneous administration of infective Dictyocaulus viviparus larvae to guinea pigs did not only result in the same lung migration as was observed after natural oral infection but also conferred a marked degree of immunity to subsequent challenge (Tománek in press). The object of the present study was to determine the degree of immunity induced in guinea pigs by subcutaneous vaccination with Dictyocaulus viviparus larvae irradiated with various X-ray doses.
MATERIAL AND METHODS

*Dictyocaulus viviparus* larvae were irradiated using a TUR X-ray machine at 180 kV, 15 mA, 2.5 mm Al filtration and a dose rate 800 R/min. The total dose of radiation was 20, 30, 40 and 50 kR.

Infected *Dictyocaulus viviparus* larvae employed for immunization were obtained from faeces of experimentally infected calves. The detritus was removed by repeated decantation. After irradiation the larvae were washed three times in sterile saline supplemented with merthiolate (1 : 10 000) and centrifuged after each washing. Groups of guinea pigs were then inoculated s/c in the back with 2 doses of 5000 radiation-attenuated larvae at 21 days’ intervals. Twelve days after the second inoculation the animals were challenged orally with 5000 infective *Dictyocaulus viviparus* larvae. They were sacrificed 7 days later and examined for the presence of larvae in the lungs. Each experimental group comprised 5 guinea pigs.

RESULTS AND DISCUSSION

Judging from the numbers of larvae recovered from guinea pig lungs, the relatively highest degree of immunity was conferred by s/c inoculation of unirradiated larvae. All groups that received larvae irradiated at levels varying from 20 to 50 kR showed approximately the same immunizing effect except for the 40 kR group where the average lung burden was influenced by an extremely high lung burden in one animal. The results are presented in Table 1.

Although no unequivocal differences could be found among groups immunized with larvae irradiated with various X-ray doses it seems reasonable to suggest that *Dictyocaulus viviparus* larvae employed for subcutaneous inoculation should be attenuated by exposure to less than 40 kR which was the X-ray dose used by Poynter et al. (1960) for oral immunization of guinea pigs. From the results obtained here after subcutaneous immunization with unirradiated larvae, it appears in any case that the migratory route does not play a role in the production of protective antibody in guinea pigs.

The preliminary experiments on guinea pigs reported herein were designed as a basis for possible subcutaneous vaccination of cattle with radiation-attenuated vaccines.

Table 1. The numbers of larvae recovered from guinea pig lungs 7 days after challenge of guinea pigs immunized s/c with X-radiation-attenuated *Dictyocaulus viviparus* larvae

<table>
<thead>
<tr>
<th>Total X-ray dose</th>
<th>No. of animals</th>
<th>No. of larvae in lung</th>
<th>Average lung burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 kR</td>
<td>4*</td>
<td>156</td>
<td>39</td>
</tr>
<tr>
<td>40 kR</td>
<td>4*</td>
<td>452</td>
<td>113</td>
</tr>
<tr>
<td>30 kR</td>
<td>5</td>
<td>187</td>
<td>37.4</td>
</tr>
<tr>
<td>20 kR</td>
<td>4*</td>
<td>121</td>
<td>30.2</td>
</tr>
<tr>
<td>unirradiated</td>
<td>5</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>nonimmunized</td>
<td>4*</td>
<td>992</td>
<td>248</td>
</tr>
<tr>
<td>controls</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* – one guinea pig died in the course of the experiment.
REFERENCES


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TOMÁNEK J. Production of immunity to *Dictyocaulus viviparous* by the subcutaneous administration of infective larvae in guinea pigs. Helmithologia — in press.


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