Short Communication

NEURAMINIDASE EFFECT ON THE GROWTH OF A TRANSPLANTABLE NICKEL SULFIDE-INDUCED RAT TUMOR

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(Received: May 15, 1978)

SUMMARY: Exposure of a weakly immunogenic nickel sulfide-induced Fischer rat tumor to Vibrio cholerae neuraminidase inhibited tumor growth in normal recipients. These recipients were found to be more resistant to a subsequent tumor inoculation.

Several studies have shown that treatment of viable tumor or normal cells with Vibrio cholerae neuraminidase (VCN) renders them more immunogenic (Sanford, 1967; Currie and Bagshawe, 1969; Lundgren and Simmons, 1971; Simmons et al., 1971a). Tumor cells exposed to VCN in vitro generally fail to grow in normal immunocompetent recipients (Sanford, 1967; Currie and Bagshawe, 1969; Simmons et al., 1971a). These tumor recipients, however, were rendered immune to a further challenge with the same tumor, the response being immunospecific (Simmons et al., 1971a, b; Rios and Simmons, 1973; Kollmorgen et al., 1976).

In this study a weakly immunogenic nickel sulfide (Ni₃S₂)-induced fibrosarcoma was employed to determine its immunostimulatory potential after treatment with VCN. Attempts to immunize these rats with subthreshold doses of live tumor cells or by excision of tumor grafts met with variable success.

The animals used were highly inbred female Fischer (F344) rats which were 8–10 weeks’ old at the time of experimentation. The tumor used was in its sixth transplant generation. This tumor was induced by intramuscular injection of Ni₃S₂ according to the method described previously (Abandowitz and Basrur, 1973). The tumor was then passaged and maintained in syngeneic rats in order to preserve its homogeneity. In untreated normal rats, an inoculation of 10³ cells killed 100% of the recipients. No spontaneous regressions were observed while the tumor was maintained.

Tumor tissue from a subcutaneous solid tumor approximately 2 cm in diameter was obtained aseptically and minced in a petri dish with cold (4 C) Hanks' balanced salt solution (HBSS). The mixture was centrifuged at 1,200 rpm for 10 min, the supernatant discarded and the tumor mince transferred to an
TABLE 1
Tumor growth of neuraminidase-treated and untreated Ni3S2 tumor cells in syngeneic Fischer rats

<table>
<thead>
<tr>
<th>First injectiona</th>
<th>Tumor incidence</th>
<th>Mean tumor diameterb (mm±SD)</th>
<th>Second injectionc</th>
<th>Tumor incidence</th>
<th>Mean tumor diameterb (mm±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCN-treated cells</td>
<td>0/4</td>
<td>0.0</td>
<td>Untreated cells</td>
<td>2/4</td>
<td>12.9±1.8</td>
</tr>
<tr>
<td>Untreated cells</td>
<td>4/4</td>
<td>37.0±1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td></td>
<td></td>
<td>Untreated cells</td>
<td>4/4</td>
<td>30.5±3.6</td>
</tr>
</tbody>
</table>

a. Tumor cell suspensions were exposed to PBS alone or to neuraminidase at 50 IU/5×10⁶ cells in 1 ml PBS, pH 7.2, at 37°C for 30 min. Tumor cell viability before treatment was 89%; after treatment, it was 88%. Rats were each injected with 1×10⁶ viable, treated or untreated cells/0.1 ml HBSS.
b. Mean tumor size includes only recipients whose tumor was recorded on day 32.
c. Rats were challenged on day 32, each with 5×10⁵ untreated tumor cells in 0.1 ml HBSS.

Erlenmeyer flask containing 20 ml sterile 0.125% trypsin and stirred on a magnetic stirrer for 30 min. The cell mixture was then poured through sterile cotton gauze and 0.25 ml fetal calf serum was added to the suspension to neutralize the trypsin. The cell suspension was then centrifuged, the cells washed four times in cold HBSS and the cell number and viability assessed by the trypan blue dye exclusion method (Boyse, Old and Chourovlinkov, 1964).

The washed tumor cells concentrated by centrifugation were then suspended in phosphate buffered saline (PBS), pH 7.2. An aliquot of this suspension was exposed to VCN (Sigma Chemical Co., St. Louis, MO, U.S.A.) at a concentration of 50 IU/5×10⁶ cells/ml PBS at 37°C for 30 min and stirred gently. The time of exposure and concentration of enzyme were based on other studies which indicated these conditions produced maximum immunogenicity (Sanford, 1967; Currie and Bagshawe, 1969). Tumor cells suspended in PBS alone served as controls. After incubation, the tumor cells were cooled rapidly and washed three times with HBSS, and 1×10⁶ viable cells in 0.1 ml HBSS were inoculated subcutaneously into the left hind leg of two groups of four Fischer rats. One extra group of rats was not initially injected with tumor cells. These rats served as the control group at the time of the second injection.

The growth of the tumors in all rats was followed by palpation and regular recordings of two-dimensional measurements taken with Vernier calipers. The VCN-tumor-immunized group of rats, remaining free of tumors for 32 days, was rechallenged subcutaneously in the opposite hind leg, each with 5×10⁵ freshly harvested viable untreated tumor cells. The control group of rats, however, was not rechallenged at this time because of the likelihood of tumor ulceration. Therefore, a second control group of rats was included and challenged. All animals were again observed for 32 days.
When VCN was used to treat the tumor cells, the enzyme appeared to have had no significant effect on cell viability and rats inoculated with such cells did not develop tumors from the immunizing injection (Table I). This treatment rendered 50% of the rats resistant to a subsequent challenge with untreated viable tumor cells (Table I). Where tumors did appear in two VCN-tumor-immunized rats, their time of appearance was delayed by 8 and 11 days and their growth was retarded. In the control group of rats, tumors were palpable in all rats 4 days after injection. All rats were killed on day 32 after rechallenge. At this time, the size of the two tumors in the VCN-tumor-immunized rats measured 12.9±1.8 mm, whereas in the four control rats, the tumors averaged 30.5±3.6 mm in diameter.

The results of this study show that immunologically competent Fischer rats inoculated with a high dose of VCN-treated tumor cells did not develop tumors. When these rats were subsequently rechallenged with untreated tumor cells, they were able to inhibit or delay the appearance of palpable tumors. Preliminary results also indicate that VCN-treated tumor cells grow in immunosuppressed rats, and that treatment of tumor cells with heat-inactivated VCN does not render them more immunogenic. Results similar to the present investigation were obtained with various other tumor-cell types exposed to VCN in vitro (Currie and Bagshawe, 1969; Simmons et al., 1971a, b; Kollmorgen et al., 1976). Other studies not employing transplantable chemical-induced tumors suggest variable responses to VCN-immunotherapy. Froese, Berczi and Sehon (1974) observed tumor enhancement in the B16 melanoma system. Sedlacek, Meesmann and Seiler (1975) noted tumor regression and enhancement in a spontaneous dog mammary carcinoma model.

The mechanism by which tumor immunogenicity is increased by VCN is not fully understood. Some reports (Sanford, 1967; Currie and Bagshawe, 1969; Bekesi, St. Arneault and Holland, 1971; Bekesi et al., 1972) suggest that VCN unmasks the surface antigens by removing sialic acid from the tumor-cell surface. The removal of the negatively charged sialic acid, however, may also allow a more rapid redistribution of cell surface antigens whereby tumor cells may become more readily recognizable by the host’s immnosurveillance.

The findings reported here provide preliminary evidence of the potential of VCN enhancement in tumor immunogenicity. In order to evaluate critically this phenomenon, other VCN-tumor dose level studies are required.

Acknowledgement

This investigation was supported in part by a grant-in-aid from the NCI Canada. Sincere thanks are expressed to Professor H. D. Geissinger for advice.
REFERENCES


