Antitumor Efficacy of Vaccinia Virus-modified Tumor Cell Vaccine

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ABSTRACT

The antitumor efficacies of vaccinia virus-modified tumor cell vaccines were examined in murine syngeneic MH134 and X5563 tumor cells. UV-inactivated vaccinia virus was inoculated i.p. into C3H/HeN mice that had received whole body X-irradiation at 150 rads. After 3 weeks, the vaccines were administered i.p. 3 times at weekly intervals. One week after the last injection, mice were challenged i.p. with various doses of syngeneic MH134 or X5563 viable tumor cells. Four methods were used for preparing tumor cell vaccines: X-ray irradiation; fixation with paraformaldehyde for 1 h or 3 months; and purification of the membrane fraction. All four vaccines were effective, but the former two vaccines were the most effective. A mixture of the membrane fraction of untreated tumor cells and UV-inactivated vaccinia virus also had an antitumor effect. These results indicate that vaccine with the complete cell structure was the most effective. The membrane fraction of UV-inactivated vaccinia virus-absorbed tumor cells was also effective. UV-inactivated vaccinia virus can react with not only intact tumor cells but also the purified membrane fraction of tumor cells and augment antitumor activity.

INTRODUCTION

TATA have been demonstrated in various tumor systems (1), but they usually have only weakly immunogenic effects on syngeneic tumors. Many attempts have been made to augment the immunogenicity of tumors (2-9). Lindenmann and Klein (10) found that influenza virus rendered the host resistant to Ehrlich ascites tumor after inducing destruction of the tumor cells. Wallack et al. reported that VOV was useful in the treatment of human cancer (11-13). Hamaoka et al. induced cytotoxic T-lymphocyte production against a syngeneic tumor in mice by priming with a hapten followed by immunization with hapten-conjugated tumor cells (14-16). We examined the effect of use of VV to modify TATA in the model described by Hamaoka et al. and succeeded in inducing resistance to MH134 and X5563 syngeneic tumors in C3H/HeN mice (17, 18). Then we demonstrated that UV-VV absorption alone was as effective as VV infection (19). In a preliminary study, we compared the antitumor effects of UV-VV TCV and VOV, finding that UV-VV TCV was the more effective (20). In the present study, we examined the antitumor effects of four different preparations of UV-VV-modified TCV.

MATERIALS AND METHODS

Experimental Animals. Female C3H/HeN mice were purchased from Charles River Laboratory, Kanagawa, Japan, and used at 6-8 weeks of age.

RESULTS

Antitumor Effect of UV-VV TCV. Two tumor cell lines, MH134 and X5563, were used. Each type of vaccine was prepared from both MH134 and X5563 tumor cells. The mortality rates of subgroups of five C3H/HeN mice each after i.p. challenge with MH134 tumor cells are shown in Table 1. In the control group, all mice died when challenged i.p. with 1 x 10^5 or 1 x 10^6 tumor cells. The TLD50 was 10^5.5. In the UV-VV TCV-treated group, all mice survived i.p. challenge with 1 x 10^5 or 1 x 10^6 tumor cells, and all mice died when challenged with 1 x 10^5 tumor cells. The TLD50 was 10^5.5.
### Table 1 Comparison of mortality rates of C3H/HeN mice immunized with UV-VV TCV and PF TCV

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Challenge dose (1 × 10^5 cells)</th>
<th>No. of deaths/no. of mice inoculated</th>
<th>Mean time to death (days)</th>
<th>TLD50 (×10^6)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MH134</td>
<td>X5563</td>
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</tr>
<tr>
<td>None</td>
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<td>5/5</td>
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<td></td>
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<td>5/5</td>
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<tr>
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<td>18.2</td>
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<tr>
<td></td>
<td>6</td>
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<td>0/5</td>
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<td></td>
<td>7</td>
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<tr>
<td></td>
<td>6</td>
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<td>1/5</td>
<td>20.0</td>
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<td>7</td>
<td>5/5</td>
<td>5/5</td>
<td>18.4</td>
</tr>
<tr>
<td>PF TCV(3m)</td>
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<td>1/5</td>
<td>4/5</td>
<td>23.0</td>
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<tr>
<td></td>
<td>4</td>
<td>1/5</td>
<td>1/5</td>
<td>24.0</td>
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<td>5</td>
<td>1/5</td>
<td>1/5</td>
<td>20.0</td>
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<td></td>
<td>6</td>
<td>1/5</td>
<td>3/5</td>
<td>18.0</td>
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<td></td>
<td>7</td>
<td>5/5</td>
<td>5/5</td>
<td>17.8</td>
</tr>
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</table>

**Immunization Schedule**

- X-ray (150 rads)
- UV-VV i.p.
- (vaccine preparing from 10^7 tumor cells)
- challenge i.p. (1 × 10^3 — 1 × 10^7 viable tumor cells)
- on the same day
- 1 W
- 1 W
- 8 W
- observation

**Fig. 1.** Immunization schedule. (a) immunized group. (b) control group. For details, see "Materials and Methods."

The mortality rates after i.p. challenge with X5563 tumor cells are also shown in Table 1. The TLD50 values of the control group and UV-VV TCV-treated group were 10^0.5 and 10^0.6, respectively.

**Antitumor Effect of PF TCV.** The mortality rates after i.p. challenge with MH134 tumor cells are shown in Table 1. The TLD50 values of the groups treated with PF TCV(1h) and PF TCV(3m) were 10^0.4 and 10^0.5, respectively. The mortality rates after i.p. challenge with X5563 tumor cells are also shown in Table 1. The TLD50 values of the groups treated with PF TCV(1h) and PF TCV(3m)-treated groups were 10^0.4 and 10^0.5, respectively. The antitumor activity of PF TCV(1h) was almost the same as that of UV-VV TCV. As judged by mortality rates, the antitumor activity of PF TCV(3m) was less than that of PF TCV(1h) against X5563 (P < 0.05 by Fisher's statistical analysis). The antitumor activity of PF TCV(3m) was less than that of PF TCV(1h) against X5563 (P < 0.05 by Fisher's statistical analysis). The differences in the antitumor activity of the groups treated with UV-VV Mfr and Mfr, UV-VV Mfr and UV-VV(s.c.) + Mfr(i.p.), UV-VV + Mfr and UV-VV + Mfr and UV-VV(s.c.) + Mfr(i.p.), respectively, were all significant at P < 0.05 by Fisher's statistical analysis.

**Comparative TLD50.** The cTLD50 is the ratio of the TLD50 in a vaccine-treated group to that in the control group and is an index of the antitumor efficacy of a vaccine that can be used to compare the effectiveness of vaccines in different experiments (25). The cTLD50 values of the vaccines tested in this work are shown in Table 3.

**DISCUSSION**

Previous studies showed that vaccinia virus-modified tumor cell vaccine induced tumor-specific resistance to MH134 and X5563 tumors. The specificity of resistance in vivo was demonstrated by cross-challenge with these two tumor cell lines; resistance was observed only when the same tumor cells were used for immunization and challenge (17). Responses of cytotoxic T-lymphocytes to X5563 and MH134-specific antibody in vitro were also reported (16).

In this study, UV-inactivated vaccinia virus-modified tumor cell vaccine was prepared by four methods, and the antitumor effects of the preparations were examined using the cTLD50 values and mortality rates as indices of effects. UV-VV TCV and PF TCV(1h) were the most effective. These two vaccines had been treated by X-ray irradiation and fixation with paraformaldehyde, respectively. In these vaccines, the cell structure is complete, and VSA was detected on the cell surface (data not shown). VSA becomes distributed over the entire cell surface on fusion of the virus envelope with the plasma membrane (26). The M, 32,000 VSA is highly immunogenic against mice (27). We observed cocapping of VSA and MH134-specific
VACCINIA VIRUS-MODIFIED TUMOR CELL VACCINE

Table 2 Comparison of mortality rates of C3H/HeN mice immunized with Mfr, UV-VV Mfr, UV-VV + Mfr, and UV-VV (s.c.) + Mfr (i.p.)

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Challenge dose (1 × 10⁶ cells)</th>
<th>No. of deaths/no. of mice inoculated</th>
<th>Mean time to death (days)</th>
<th>TLD₅₀ (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MH134</td>
<td>X5563</td>
<td>MH134</td>
<td>X5563</td>
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<td>None</td>
<td>1</td>
<td>3/5</td>
<td>2/5</td>
<td>2.0</td>
</tr>
<tr>
<td>Mfr</td>
<td>2</td>
<td>4/5</td>
<td>4/5</td>
<td>2.5</td>
</tr>
<tr>
<td>UV-VV Mfr</td>
<td>3</td>
<td>0/5</td>
<td>0/5</td>
<td>2.0</td>
</tr>
<tr>
<td>UV-VV + Mfr</td>
<td>4</td>
<td>0/5</td>
<td>0/5</td>
<td>2.0</td>
</tr>
<tr>
<td>UV-VV (s.c.) + Mfr (i.p.)</td>
<td>5</td>
<td>0/5</td>
<td>0/5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Table 3 Comparison of cTLD₅₀ values (10⁶) of tumor cell vaccines

<table>
<thead>
<tr>
<th>Immunization</th>
<th>MH134</th>
<th>X5563</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-VV TCV</td>
<td>6.0</td>
<td>6.1</td>
</tr>
<tr>
<td>PF TCV(1h)</td>
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<td>PF TCV(3m)</td>
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<td>UV-VV Mfr</td>
<td>4.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Mfr</td>
<td>0.8</td>
<td>2.9</td>
</tr>
<tr>
<td>UV-VV + Mfr</td>
<td>4.4</td>
<td>4.9</td>
</tr>
<tr>
<td>UV-VV(s.c.) + Mfr(i.p.)</td>
<td>0.8</td>
<td>0.8</td>
</tr>
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</table>

surface antigen (data not shown). In UV-VV TCV and PF TCV(1h), VSA seems to be closely associated with TATA on the membrane of cells whose cell structure is not destroyed. We suppose that this situation is advantageous for interaction of the antigen-presenting cell with the cell membrane. In a preliminary study, we found that the cTLD₅₀ values of VOV challenged with MH134 and X5563 were 10⁴.9 and 10⁴.8, respectively (20). VOV, which was a preparation of sonicated, VV-infected tumor cells containing all the fractions of VV-infected tumor cells, and UV-VV Mfr, which was a purified membrane fraction of UV-VV-absorbed tumor cells, had similar effects. This finding suggests that the effective fraction of UV-VV-modified TCV is the membrane fraction.

UV-VV Mfr and UV-VV + Mfr had almost the same effect, but UV-VV(s.c.) + Mfr(i.p.) had no effect. UV-VV Mfr was a membrane fraction of UV-VV-absorbed tumor cells and contained VSA (data not shown), the association of which with TATA would result in augmentation of immunogenicity against tumor cells. Mfr was a membrane fraction of untreated tumor cells. The UV-VV + Mfr-treated group received an i.p. injection of Mfr; thus there was no association between VSA and TATA. Therefore, our findings suggest that the association of VSA with TATA was essential for augmenting the antitumor activity. Lindenmann and Klein (10) found that merely mixing egg-grown virus with homogenates of uninfected tumor cells did not augment the immunogenicity of the tumor cell extracts, thus excluding the possibility that the virus acts simply as an adjuvant. The mechanism by which a virus augments the immunogenicity of TATA is unknown but has been suggested to be mainly by acting as a helper antigen (28). VSA exists in close proximity to TATA and during the process of response to VSA, the host immune system responds more strongly to adjacent TATA than in the absence of VSA. We found that UV-VV + Mfr had the same effect as UV-VV Mfr, clearly indicating that VSA acted as a helper antigen not only on intact tumor cells but also on membrane fragments.

VSA in close association with TATA presumably augments the immunogenicity of TATA on the intact cell structure. Previously, we demonstrated that infection of murine or human tumor cells with VV induced the cells to react via the alternative pathway (29, 30). The activation of the alternative complement pathway induced by VV or UV-VV occurred only at the surface of VV-infected or UV-VV-absorbed tumor cells. Therefore, the intact cell structure is essential for activation of the alternative complement pathway and recognition of the helper antigen by the antigen-presenting cell. Paraformaldehyde-fixed vaccine is reported to be effective for inducing a humoral and cellular immune reaction against human immunodeficiency virus 1 (31). Our results also showed that fixation with paraformaldehyde for 1 h did not affect the antitumor activity.

In a preliminary experiment, we used this protocol in tumor-
bearing mice. The mice were inoculated i.p. or s.c. with $1 \times 10^3$
MH134 tumor cells, and beginning 1 week later, UV-VV TCV
was injected i.p. three times at weekly intervals. However, all
the mice finally died of tumors. Healthy mice and tumor-
bearing mice showed quite different augmentations of antitu-
mor immunity. We think this immune protocol would be useful
for preventing metastasis after surgical operation with nonspe-
cific adjuvant therapy.

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30. Koymats, H., Sendo, F., Immunogenicity of viable xenogenized tumor
31. Zugury, D., Bernard, J., Cheynier, R., Desportes, I., Leonard, R., Fournier,
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Goussard, B., Dechazal, L., Burny, A., Nara, P., and Gallo, R. C. A group
specific anamnestic immune reaction against HIV-1 induced by a candidate
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