BRIEF COMMUNICATION

Route of Immunization and the Therapeutic Impact of Recombinant Anticancer Vaccines

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Active immunization with recombinant poxviruses, e.g., recombinant vaccinia virus (rVV), containing a variety of experimental tumor-associated antigens can result in prolonged survival of animals bearing established tumors (1-3). Poxviruses are currently being tested in patients with cancer [Rosenberg SA, et al.: unpublished data; (4)]. The first recombinant to be employed was vaccinia virus, which demonstrated its safety and efficacy in the worldwide campaign to eliminate smallpox (5). Another category of poxviruses, avipox virus, is currently being explored as a vector for the immunotherapy for cancer and infectious disease. These viruses can cause slow-spreading pox diseases in birds but do not productively replicate in mammalian cells and thus are being investigated as a safer alternative to rVV for immunization (6).

Since the early days of the war on smallpox, the traditional route of immunization has been scarification, and recent clinical trials with recombinant vaccinia viruses have followed suit. As it is currently done, scarification involves the use of a bifurcated needle to make multiple small and superficial incisions in the skin onto which a drop of inoculum is placed and allowed to absorb into the site. As advocated by the World Health Organization, the safety and efficacy of scarification as a route of administration of vaccinia virus has been demonstrated in millions of people.

Vaccination against cancer and infectious diseases with the use of recombinant viruses can also be accomplished via scarification (1,7). Our own early attempts to generate primary immune responses against vaccinia viral antigens by the use of this method of immunization, however, were unsuccessful. To explore whether the route of immunization affected the efficacy of tumor treatment, we used the experimental murine colon adenocarcinoma (CT26.WT) transduced with the gene for the model TAA, β-galactosidase (β-gal), designated CT26.CL25. Previously, Wang et al. (3) have shown that recombinant fowlpox virus (rFPV) expressing β-gal when injected intravenously can specifically reduce the number of pulmonary nodules in mice bearing 3-day established tumor metastases. Likewise, rVV can also mediate partial therapeutic responses in some cases (2). Active treatment of pulmonary metastases with the use of rVV or rFPV was enhanced by the systemic administration of interleukin 2 (2).

BALB/c mice were given intravenous injections of CT26.CL25 (β-gal+) tumor cells. After 3 days, tumor-bearing mice were administered rVV expressing β-gal or a control vaccinia virus expressing nucleoprotein from the influenza virus by use of either intravenous, intramuscular, subcutaneous, or intradermal tail scarification routes of injection. Nine days later, lungs were harvested and grossly visible metastases present on the surface of the lungs were enumerated in a coded, blinded fashion as previously described (2,3,8). Fig. 1 represents the pooled averages of three experiments done with the same protocol. As shown in Fig. 1, injection of the control rVV, V69, regardless of the route of immunization, had little to no effect on the number of tumor nodules when compared with the no-treatment group. By contrast, all of the mice that received the rVV expressing β-gal, VJS6, had a statistically significantly reduced number of pulmonary metastases compared with untreated mice (overall mean ± standard error [SE], 244 ± 9.5 metastases or mean by experiment, >250, 233, and >250, respectively). The mice that were immunized with VJS6 intravenously (overall mean ± SE, 20 ± 13.2 metastases or mean by experiment, 11, 0.2, and 49, respectively) showed a statistically significantly enhanced reduction in the average number of metastases compared with either subcutaneously (overall mean ± SE, 123 ± 43 metastases or mean by experiment, 154, 25, and 191, respectively) or intradermal tail scarification (overall mean ± SE, 143 ± 47 metastases or mean by experiment, 150, 84, and 191) routes of injection. The mice that received VJS6 intramuscularly had an overall mean ± SE of 46 ± 37.6 metastases (mean by experiment, 56, 11, and 72, respectively) that was not statistically different from VJS6 immunization by the subcutaneous or the intradermal tail scarification route when an adjustment was made for the number of tests accomplished. Both the intravenous and the intramuscular routes consistently demonstrated statistically significant reductions in the numbers of metastases compared with the no-treatment group in all three experiments. Together, these experiments indicated that it may be more efficacious to immunize by the use of either the intravenous or the intramuscular route of injection.

In these studies, rVV alone mediated an antitumor effect. This observation is in apparent conflict with an earlier report from our laboratory (2). Previously, Bronte et al. (2) used a crude preparation consisting of a cellular lysate of virally infected BSC-1 monkey kidney cells. However, the method of viral processing has been significantly improved since this paper was published. Now, crude lysates are purified over a sucrose cushion, thus reducing the presence of cellular membrane and cytosolic and nuclear components (9). Furthermore, purification of the virus reduces the induction of irrelevant immune responses against these additional elements within the crude lysate. This procedure may also eliminate potentially immunosuppressive molecules present in the cellular preparation.

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Active immunization with the non-replicating rFPV expressing the tumor-associated antigen β-gal (rFPV.bg40k) also demonstrated a similar dependency on the route of injection. Fig. 2 represents the pooled averages of four experiments done with the use of the same 3-day lung metastases protocol. All of the mice that received rFPV.bg40k showed a significant reduction in the number of metastases compared with the no-treatment group (overall mean ± SE, 239 ± 27 metastases or mean by experiment, >250, >250, >250, and 208, respectively). The intravenous route (overall mean ± SE, 53 ± 26 metastases or mean by experiment, 32, 35, 53, and 91, respectively) was shown to be superior to the intramuscular route (overall mean ± SE, 150 ± 41 metastases or mean by experiment, 115, 195, 201, and 90, respectively) or the subcutaneous route (overall mean ± SE, 174 ± 42 metastases or mean by experiment, 217, 219, 167, and 94, respectively). The intradermal tail scarification route was not performed in these studies. We have previously demonstrated that wild-type FPV has no effect on the tumor burden in the same 3-day lung metastases model (3). These findings suggest that the intravenous route of rFPV.bg40k immunization may be superior to the others examined.

These data comprise a comparison of the effect of different routes of immunization on the active treatment of malignant disease. As opposed to vaccination for the prevention of viral infections like smallpox, the active immunization of cancer may require the use of much higher doses of recombinant viruses. Since it is difficult to attain viral titers of more than 10^10 plaque-forming units (pfu)/mL, the quantity of immunogen that can be administered by the use of scarification is limited because only small volumes (10-20 μL) are generally used. In addition, the actual volume delivered is unknown, since not all of the viral material placed at the site of scarification is adsorbed. It was thus important to determine other routes that more efficiently delivered the recombinant viruses.

On the basis of these findings, we conclude that the intravenous and intramuscular routes for the injection of pox-viruses have enhanced efficacy for the active treatment of cancer. These results are in accord with previous studies by Andrew et al. (7), who demonstrated that an intravenous injection of a recombinant vaccinia virus stimulated the highest antibody and cytotoxic T-cell responses to the heterologous product when compared with intraperitoneal, intranasal, footpad, and tail scarification routes. The intravenous route may be most effective because the virus, once systemic, can infect a broader number of cells.
cells, particularly those of the reticuloendothelial system, compared with the more local nature of subcutaneous, intramuscular, or intradermal tail scarification immunizations. For safety reasons, rVV may be best injected intramuscularly to decrease the possibility of a systemic infection, whereas with a nonreplicating virus such as rFPV, intravenous inoculation may be safer and more efficacious.

However, as with all data derived from animal models, these findings must be applied to the clinic with caution. Specifically, vaccinia virus may have a significantly greater replication potential in human skin compared with its murine counterpart. Clinical trials already under way in patients with malignant melanoma may yield more definitive conclusions.

References


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