Re: Cytosine Deaminase/5-Fluorocytosine-Based Vaccination Against Liver Tumors: Evidence of Distant Bystander Effect

We recently reported that suicide vaccination by subcapsular injection of autologous tumor cells expressing the bacterial gene cytosine deaminase followed by 5-fluorocytosine (5-FC) treatment significantly increased the survival of rats carrying a single liver metastasis (1). We wish to supplement these data with results concerning the effect of suicide vaccination in a polymetastatic liver model.

DHD/K12/PROb (PROb) rat colon carcinoma cells (20.0 × 10^6) (2) were injected in the portal veins of 27 male BDIX rats (IFFA CREDO, L’Arbresle, France) to generate experimental disseminated liver metastasis. Five days later, the presence of multiple liver tumors was confirmed surgically, and the animals were randomly assigned to one of three groups. In the control group (n = 11), animals were left untreated. In the first experimental group (n = 10), 1.5 × 10^6 PROb cells expressing the cytosine deaminase gene (PRObCD cells) (1) were injected subcapsularly in the left lobe. To explore if homing of circulating suicide cells to pre-existing tumors might improve the vaccination efficiency, we injected 10.0 × 10^6 PRObCD cells into the portal veins of a second experimental group (n = 6) of animals. After 24 hours, rats of the experimental groups received three daily (7 days per week) intraperitoneal injections of 5-FC (Produits Roche, Fontenay Sous Bois, France) (800 mg/kg of body weight) for 30 days, followed by daily 5-FC injections (5 days per week) for 3 months. 5-FC was dissolved in saline at 15 mg/mL. All of the surgical procedures and the care given to the animals were in accordance with institutional guidelines.

Survival curves for each group are presented in Fig. 1. All control animals died between 47 and 157 days, after the injection of the PROb cells (median = 95 days). In the subcapsular vaccination group, the median survival of vaccinated animals was increased by 58% (150 versus 95 days), and four of 10 rats were still alive at day 210. According to the log-rank test, this vaccination increased the survival of these animals (two-sided P = .01). For the second experimental group (intraportal vaccination), the median survival was 122 days, and one of six animals was still alive at day 210 (two-sided P = .04). Despite the fact that this latter result was not statistically significant (P = .025 used as the criterion of statistical significance), it is noteworthy that intraportal injection of a large number of autologous suicide tumor cells did not further stimulate disease progression. In all groups, dead animals generally exhibited advanced peritoneal carcinomatosis, and some of them had pulmonary metastasis (control group 445%; subcapsular vaccination group 416%; portal vein vaccination group 420%).

Recent studies (3–5) reported the use of a vaccinia viral vector to introduce the cytosine deaminase gene into tumors in different animal models. Although highly specific delivery of the gene to the tumor was observed, the immune response raised against the vector was shown to limit the period of cytosine deaminase gene expression (5). In this context, vaccination with autologous suicide tumor cells that are efficiently controlled by cytosine deaminase-catalyzed conversion of nontoxic 5-FC to toxic 5-fluorouracil can be considered a valuable alternative strategy in the treatment of disseminated liver metastasis.

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NOTES

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Fig. 1. Survival of rats bearing disseminated liver tumors after vaccination. Rats (n = 27) were given an injection of 2.0 × 10^6 PROb rat colon carcinoma cells in the portal vein. Five days later, the presence of multiple tumors was confirmed surgically, and animals were randomly assigned to one of three groups. During the surgical procedure, 16 of these rats were vaccinated by the injection of PROb rat colon carcinoma cells transfected with a vector encoding cytosine deaminase (PRObCD cells). In the subcapsular vaccination group (n = 10), 1.5 × 10^6 PRObCD cells were injected under the liver capsule in the left lobe. In the intraportal vaccination group (n = 6), 10.0 × 10^6 PRObCD cells were injected in the portal vein. After 24 hours, rats of the vaccinated groups were treated with 5-fluorocytosine for up to 4 months, as described in the text. The survival curves of each group of rats, determined by Kaplan–Meier analysis, are presented. Survival probability is shown as a function of days after injection of PROb cells. The 95% confidence intervals (95% CI) and the number of animals at risk are shown for vaccinated and control rats at 120 and 180 days. Bold solid line = no treatment; solid line = intraportal vaccination; dashed line = subcapsular vaccination.