Immunotherapy of the rat 13762SC mammary adenocarcinoma by vaccinia virus augmentation of tumor immunity

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(Received 9 August 1989; accepted 9 May 1990)

We studied whether vaccinia virus (VV) functioned as an immunogenic carrier in augmenting anti-tumor immunity in rats bearing a syngeneic metastatic tumor. The primary tumor was induced by injecting $10^6$ 13762SC mammary adenocarcinoma cells subcutaneously into the right hind footpad of Fischer 344 rats. A concomitant anti-tumor response is induced by the tumor as demonstrated by the inhibited growth of a second tumor challenge given in the contralateral footpad 3–15 days later. Attempts were made to increase the concomitant immunity by injecting tumor-bearing animals intramuscularly with irradiated, VV-infected or uninfected 13762SC cells without adjuvant. Provided the immunotherapy was done within 5 days of the tumor challenge, administration of $10^6$–$10^7$ irradiated, VV-infected 13762SC cells resulted in significantly slower tumor growth, or led to complete tumor regression, compared to control animals given no treatment. In contrast, tumor growth in animals given only VV or given irradiated, uninfected 13762SC cells, alone or mixed with VV, was the same as that in control animals. Kinetics of early primary tumor growth were predictive of a longer-term anti-tumor effect. Rechallenge of 13762SC tumor-cured animals with either the homologous or with a heterologous syngeneic mammary adenocarcinoma showed the animals to be specifically 13762SC tumor-resistant, since only rats challenged with the heterologous mammary adenocarcinoma developed progressive tumors. We interpret these results to mean that early immunotherapy with irradiated, VV-infected 13762SC cells enhances an on-going anti-tumor immune response sufficiently to cause rejection of the primary tumor and any metastases that have occurred. We also believe that later immunotherapy with irradiated, VV-infected cells has no effect due to tumor-induced immunosuppression becoming paramount.

Introduction

The goal of cancer immunotherapy is to produce effective anti-tumor resistance through modification of the host response, thereby causing either prolonged survival or complete tumor regression. Although innate and adaptive immune mechanisms both contribute to tumor resistance, adaptive tumor immunity is the most important mechanism because it is systemic and involves a memory response [33]. While innate tumor immunity can be mediated by macrophages [9] and natural killer cells [15], adaptive tumor immunity is mediated primarily by T cells [2, 7, 15, 24].

One potential method for augmenting the anti-tumor response is increasing the immunogenicity of tumor cells through xenogenization; i.e. through provision of additional helper determinants on the cell surface. If a strong immunogen is...
recognized in association with weak tumor immunogens, the immune response against the tumor cell may be enhanced. Using viruses to provide these helper determinants for the response against tumor-associated transplantation antigens (TATAs) dates to 1967, when Lindenmann and Klein [25] showed that peritoneal membranes debris from virus-cured, tumor-bearing mice would protect naive mice from challenge with the same ascites tumor cells, while membranes from uninfected tumor cells would not. Many workers have confirmed this finding, demonstrating that membranes and/or oncolysates from virus-infected tumor cells induce anti-tumor immunity [1, 3, 4, 16, 37, 40].

Most studies of virus-augmented tumor immunity have dealt with non-metastatic tumors and have involved immunizations prior to tumor challenge. The work we describe here differs in both regards. First, the syngeneic 13762SC mammary adenocarcinoma used is highly metastatic. Subcutaneous challenge with 10^6 tumor cells in the hind footpad of Fisher 344 rats results in a progressive primary tumor which quickly metastasizes through the single draining popliteal lymph node in the leg. Second, we have studied the effects of a single treatment with irradiated, vaccinia virus (VV)-infected tumor cells administered at various times after tumor induction. No drugs or adjuvants were used.

Our choice of VV for these studies was based upon the ability of this virus to insert upwards of a dozen different proteins into the plasma membrane of infected cells [8, 26, 27, 34, 42]. We reasoned that this large number of virus-specified proteins embedded in the surface of VV-infected 13762SC tumor cells would have a high probability of providing the helper determinants needed for augmenting the immune response to tumor-specific surface antigens [4]. In this report we show that rats bearing syngeneic 13762SC tumors can experience complete primary tumor regression and complete clearance of metastases. This occurs provided irradiated, VV-infected tumor cells are given soon after tumor challenge, during the time that we believe the level of anti-tumor immunity is increasing, but before tumor-induced suppression becomes dominant. Such animals were found to possess a long-lasting, tumor-specific immune resistance.

Materials and methods

Animals

All experiments were initiated with 8–10-week-old 100–125 g female Fischer 344 (F344) rats (Charles River Canadian Breeding Laboratories Inc., St Constant, Que.). The rats were housed in translucent, plastic solid-bottomed cages containing woodchip bedding. The rats were provided with unlimited water and standard rat chow pellets, and a 12 h light/12 h dark schedule was maintained. All rats were acclimatized to their new surroundings for at least 2 weeks before being used in an experiment. Randomly chosen animals tested negative for mycoplasma infection by serology and pathology.

Tumor cell lines

13762SC rat mammary adenocarcinoma cells were obtained from Dr Svein Carlsen (University of Saskatchewan, Saskatoon, Sask.), who derived them by adapting 13762 MAT-B cells to grow in cell culture. The 13762SC tumor cells are poorly differentiated and highly metastatic to regional lymph nodes and visceral organs [18]. 13762SC cells were cultured at 37°C in RPMI 1640 medium (Gibco