Immunotherapy of Prostate Cancer in the Dunning Rat Model: Use of Cytokine Gene Modified Tumor Vaccines


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ABSTRACT

Adenocarcinoma of the prostate is the most common cancer in men. The majority of cancers are discovered once they have already metastasized, and there is no effective therapy for prostatic cancer at this stage. The use of cytokine-secreting tumor cell preparations as therapeutic vaccines for the treatment of advanced prostate cancer was investigated in the Dunning rat R3327-MatLyLu prostatic tumor model. IL-2 secreting, irradiated, tumor cell preparations were capable of curing animals with s.c. established tumors, and induced immunological memory that protected animals from subsequent tumor challenge. Immunotherapy was less effective when tumors were induced orthotopically, but nevertheless led to improved outcome, significantly delaying, and occasionally preventing, recurrence of tumors after resection of the cancerous prostate. Granulocyte-macrophage colony stimulating factor secreting tumor cell preparations were less effective, and interferon-γ secreting cells had only a marginal effect. Induction of a potent immune response in tumor bearing animals against the nonimmunogenic MatLyLu tumor supports the view that active immunotherapy warrants further investigation as a potential therapeutic approach to prostate cancer.

INTRODUCTION

Cancer of the prostate is the most commonly diagnosed cancer in men and is the second most common cause of cancer death in Western civilization (1). Progression of prostatic cancer in humans is not accompanied by a vigorous antitumor immune response, although weak immune responses have been occasionally documented in some patients (2). Several attempts to actively immunize patients with adenocarcinoma of the prostate have shown little or no therapeutic benefit (3, 4). Thus, like most other human neoplasms, prostate cancer is largely a nonimmunogenic cancer. This, together with the demonstration that spontaneously occurring tumors in animals do not induce protective immunity (5, 6), and that no increase in tumor incidence is seen in T-cell-deficient mice (7), have led to the belief that naturally occurring tumors do not encode tumor-specific antigens capable of inducing an effective immune response, and that active immunotherapy of cancer may be futile. In a landmark study, van Pel and Boon (8) have shown that a nonimmunogenic murine tumor could be converted to an immunogenic form which was capable of inducing systemic immunity against the parental tumor. This finding demonstrated that the parental tumor did in fact encode tumor antigens, but that in its original form the tumor was incapable of presenting the tumor antigen(s) to the immune system. If this conclusion applies to human cancer as well, active immunization of patients against nonimmunogenic forms of cancer may be possible.

Rat models have been used to study prostate biology because the rat prostate has many features in common with the human prostate. In addition, spontaneous prostatic tumors have been observed in several strains of rats. The most thoroughly studied and readily available tumor is the Dunning tumor and its derivatives (9–12). Several sublines with varying degrees of hormone dependency and metastatic behavior have been isolated from the original tumor, providing a spectrum of prostatic cancers with distinctive individual properties which correspond to various stages of prostate cancer in humans. One such line, R3327-MatLyLu, is an anaplastic androgen independent tumor which spontaneously metastasizes to the lymph nodes and the lung (10). Furthermore, since the MatLyLu tumor does not exhibit any signs of intrinsic immunogenicity (see below), it constitutes an excellent model to explore immunotherapeutic protocols for the treatment of metastatic human prostatic cancer. A growing number of studies have shown that genetically engineered tumor cells expressing cytokines such as IL-2, IFN-γ, IL-4, IL-6, IL-7, or GM-CSF could immunize mice against a subsequent challenge with parental tumor cells (13–25). It was recently demonstrated that cytokine secreting, growth inactivated, tumor cell preparations were also active in tumor bearing animals, reducing tumor progression and even curing some animals of a preestablished tumor (20–24). We therefore tested whether irradiated MatLyLu tumor cell preparations genetically engineered to express either IL-2 (MAT/IL-2), GM-CSF (MAT/GM), or IFN-γ (MAT/IFN) were capable of inducing an immune response against the nonimmunogenic parental tumor, and altering the course of disease in tumor bearing animals.

MATERIALS AND METHODS

Generation of Cytokine Secreting Prostatic Tumor Cells. R3327-MatLyLu cell lines stably expressing the human IL-2, the mouse IFN-γ, or the murine GM-CSF were constructed by Rosenthal et al.3 Retroviral vectors carrying the corresponding cDNA were constructed by Wellings et al.4 Gene Modified Tumor Vaccines

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3 The abbreviations used are: IL, interleukin; IFN, interferon; GM-CSF, granulocyte/macrophage colony stimulating factor; CTL, cytotoxic T-lymphocyte.


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cell suspensions. Cell viability was determined by trypan blue dye exclusion. After trypsinization, the cells were washed 3 times in phosphate buffered saline to remove any traces of serum or media.

Prostatic tumors were induced orthotopically by injection of 5 × 10^6 tumor cells into the ventral prostatic lobe of Copenhagen rats and s.c. by implantation of 1 × 10^4 MatLyLu cells into the right hind leg of syngeneic Copenhagen rats. It was previously determined that these cell numbers lead to tumor take in 100% of the animals (27). Tumor growth was assessed biweekly by abdominal palpation and microcalipers. Immunizations were carried out 3, 6, and 9 days post-tumor implantation with 1.0 × 10^6 irradiated (5000 rads) cytokine secreting MatLyLu cell preparations injected intradermally into the left hind leg.

**Tumor Recurrence Model.** Tumors were induced intraprostatically with 1 × 10^4 cells and 4 days later the cancerous prostate was surgically removed. It was previously determined that when prostatectomy was performed 4 or more days after implantation of MatLyLu cells, tumors recurred locally in 100% of the animals, despite meticulous surgical removal of the cancerous prostatic tissue. One day following prostatectomy, treatment with 1 × 10^6 irradiated MatLyLu cells was initiated and repeated twice, 3 days apart.

Animal care was in accordance with institutional guidelines. When animals showed signs of moribidity, they were euthanized by pentobarbital overdose.

**Europium Release Assay.** Three groups or rats (3 animals/group) were immunized 3 times by i.p. injections of 5.0 × 10^6 irradiated (5000 rads) parental MatLyLu cells. Fourteen days later, pooled splenocytes were isolated and cultured for 6 days in the presence of irradiated MatLyLu cells used as stimulators. At the end of the culture period, effector cells were cocultured with several targets at the indicated effector to target ratios, and cytotoxicity was determined in a 5-h europium-diethylenetriaminepentaacelate release assay (28). Europium-diethylenetriaminepentaacelate release was measured by time resolved fluorescence (Delfia fluorometer; Wallac, Inc., Gaithersburg, MD).

**RESULTS**

Immunogenic animal tumors are not thought to be good models for human cancer (5, 6). To determine whether parental MatLyLu cells are intrinsically immunogenic, one group of rats was given i.p. injections of 5 × 10^6 irradiated MatLyLu cells once, and another group received injections 3 times at 5-day intervals. Two weeks following the third injection, rats were either challenged s.c. with 1.0 × 10^6 live MatLyLu cells (the minimal dose of cells leading to tumor formation in 100% of injected animals), or spleen cells were isolated to determine whether tumor specific CTLs were induced in the vaccinated rats. Upon challenge with unmodified MatLyLu cells, all rats developed tumors and no difference was seen in the rate of tumor growth (Fig. 1) or survival (data not shown) between vaccinated and age-matched control rats. As shown in Fig. 2, tumor specific CTLs were not detected in rats immunized with parental MatLyLu cells. Thus, the MatLyLu tumor did not exhibit any evidence of intrinsic immunogenicity as judged by the stringent criteria set forth by Hewitt et al. (5), or standard cytotoxicity assays (Fig. 2). In stark contrast to unmodified MatLyLu cells, IL-2 secreting cells induced a strong CTL response which specifically recognized parental MatLyLu cells, as well as the related R3327-G subline (9), but not the unrelated adenocarcinoma CopMeEla cell line of the same genetic background. Thus, experimental manipulation of the parental MatLyLu tumor to express IL-2 converted this nonimmunogenic tumor to an immunogenic form capable of inducing an in vivo tumor specific immune response.

To assess the therapeutic value of cytokine secreting MatLyLu cells as cellular vaccines in rats carrying an established tumor, 1.0 × 10^6 parental MatLyLu cells were injected s.c. and treatment with various tumor cell preparations was initiated 3 days later. Unless otherwise specified, treatment in this and subsequent experiments consisted of 1.0 × 10^6 irradiated cells injected intradermally, followed by 2
Since it was noted that the site of tumor implantation can greatly though they may not correspond to the anatomical origin of the tumor. These sites are easily accessible for experimental manipulation, all example), cure rates of 100% were observed.

In a comparative study, it was recently shown that GM-CSF secreting tumor cells were the most active preparation capable of inducing antitumor immunity against the nonimmunogenic, highly metastatic murine B16-F10 melanoma (24). We therefore compared the therapeutic effect of IL-2 and GM-CSF-secreting tumor cell preparations in rats carrying a 3-day-old MatLyLu tumor. As shown in Fig. 5A, treatment of s.c. implanted tumor bearing rats with IL-2 secreting cells led to complete tumor regression in all animals (5 of 5), while only 2 of 5 animals treated with GM-CSF secreting cells were cured of their preestablished tumors. No difference in the effect of IL-2 versus GM-CSF secreting tumor cell preparations was observed in rats bearing an orthotopically induced tumor (Fig. 5B). Provided that murine-derived GM-CSF is interchangeable with rat-derived GM-CSF in vivo, as is the case in vitro, our results show that in this experimental model, IL-2 secreting tumor cell preparations are equal or superior to GM-CSF secreting cells.

In order to determine whether rats exhibiting long term tumor regression (Fig. 5A) would be resistant to a second challenge with tumor cells, the “cured” rats were given s.c. injections of 5 × 10^6 MatLyLu cells (5 times the minimal tumorigenic dose). No tumors formed in rats “cured” of their original tumor by treatment with IL-2 secreting cells (5 of 5), while tumors grew at a similar rate in 2 of 2 animals “cured” by treatment with GM-CSF secreting cells, and in a control group consisting of age-matched animals (5 of 5). This result indicates that immunological memory was established in rats cured of

\[ ^3 \text{M. A. Moore, personal communication.} \]
their established tumor by treatment with inactivated IL-2 secreting tumor cell preparations. In a similar study, it was recently reported that establishment of immunological memory could be achieved using IL-2 secreting tumor cell preparations in mice cured of induced bladder tumors (23).

Local recurrence and the development of distant metastases are responsible for most therapeutic failures in human prostate cancer. To test whether cytokine secreting tumor cell preparations have a therapeutic benefit as an adjuvant therapy following surgical removal of the cancerous prostate, 1.0 x 10^6 MatLyLu cells were orthotopically implanted and 4 days later the prostate was surgically removed. Tumors recurred locally in all animals despite careful resection of the cancerous organ. One day following prostatectomy, animals were treated with various tumor cell preparations. Direct surgical inspection of the lower abdomen 10 days later revealed that tumors recurred locally in all control animals as well as in animals treated with unmodified MatLyLu cells and also, albeit significantly retarded, in animals treated with MAT/IFN or MAT/GM cells. In contrast, no macroscopic tumors were evident in rats treated with IL-2 secreting MatLyLu cells (Fig. 6A). However, in the majority of these animals (4 of 5), the residual tumor was not completely eliminated since tumors grew, albeit with a significant delay, and the rats eventually succumbed to disease (Fig. 6B).

DISCUSSION

Using an increasingly relevant animal model to explore the utility of cytokine secreting tumor vaccines for the treatment of prostate cancer, we have shown that inactivated, IL-2-secreting, tumor cells were most effective in reducing tumor growth and prolonging the survival of tumor bearing animals. We also observed that MatLyLu tumors orthotopically implanted in the prostate were less susceptible to treatment with cytokine secreting tumor cells than s.c. implanted tumors (Figs. 3 and 5). The ectopically and orthotopically implanted tumors appear to grow at a similar rate, and rats bearing a tumor at either site succumb to disease within 3.5 weeks (Figs. 3 and 5). It is,
therefore, difficult to attribute the superior effect seen in rats bearing s.c. implanted tumors to slower tumor growth at this site (although in some experiments we observed reduced tumor growth in s.c. implanted animals). As previously suggested (30, 31), it is more likely that the prostate constitutes a less permissive environment than the skin for the development of an effective antitumor immune response, highlighting the importance of using orthotopically implanted tumors to assess the effectiveness of therapeutic protocols (29). The modest though reproducible therapeutic benefit seen in animals bearing an orthotopically implanted tumor is encouraging (Figs. 3B, 5B, and 6), and suggests that additional studies may lead to improved outcome.

Growth inactivated (irradiated) MatLyLu cells were used to vaccinate animals not only to simulate a clinical setting but also to demonstrate that the parental tumor cells were devoid of intrinsic immunogenicity. The potential utility of cytokine gene modified tumor vaccines was initially demonstrated using live tumor cell preparations, which was possible because the cytokine-secreting cells lost their ability to grow in vivo, i.e., to form tumors in the animal (13-19). However, in such studies the intrinsic immunogenicity of the tumor cells could not be determined since it was unclear whether the cytokine contributed to the induction of antitumor immunity or whether its only function was to inhibit tumor growth in the animal. Indeed, it was recently shown that several tumors used in previous studies were immunogenic when administered in an unmodified, though irradiated, form, suggesting that the cytokine secreted from the live tumor cells was not responsible for the observed antitumor effect (24).

The MatLyLu prostatic carcinoma line is analogous to hormone-refractory, metastatic human prostate cancer in many ways (9-11). Using stringent testing criteria set forth by Hewitt et al. (5), we have shown that MatLyLu cells are nonimmunogenic in the rat (Figs. 1 and 2), and therefore represent a useful model for immunological studies addressing a similar situation in human cancer. The finding that genetically modified, IL-2 secreting, MatLyLu cells were capable of inducing a significant immune response against the parental tumor demonstrates that MatLyLu cells encode potent tumor rejection antigens which can be made accessible to the immune system upon proper manipulation of the tumor cells. That nonimmunogenic tumors can be
experimentally manipulated to induce an immune response against the parental tumor was first demonstrated by Van Pel and Boon (8), and subsequently extended to other tumor systems (20–24, 32). Recent studies have shown that this paradigm may also apply to peripherally expressed self-antigens, providing a plausible explanation as to the maintenance of peripheral tolerance (33, 34).

In conclusion, these observations support the view that active immunization of cancer patients, including prostate cancer patients, deserves consideration despite lack of demonstrable immunogenicity in many human tumors.

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