Neutron or Photon Irradiation for Prostate Tumors: Enhancement of Cytokine Therapy in a Metastatic Tumor Model

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ABSTRACT

We have shown that implantation of human prostate carcinoma PC-3 cells in the prostates of nude mice led to the formation of prostate tumors with metastases to para-aortic lymph nodes. We found that day 6 prostate tumors were responsive to systemic injections of interleukin 2 (IL-2) therapy. We have now investigated the combination of primary tumor irradiation and IL-2 for metastatic prostate cancer in this preclinical tumor model. The effect of neutron radiation was compared with that of photon radiation. Advanced prostate tumors (∼0.4 cm) were irradiated, and a day later, mice were treated with systemic IL-2 for three weekly cycles. In separate experiments, mice were either sacrificed on day 30 to assess prostate tumor size and tumor histology or followed for survival. A dose-dependent inhibition of prostate tumor growth was caused either by photons or neutrons, but neutrons were more effective than photons with a relative biological effectiveness of 2. The tumor inhibition obtained with 250 cGy neutrons and 500 cGy photons was significant (>75%) and was further increased (≥90%) by addition of IL-2 therapy. In survival studies, the combination of radiation and IL-2 showed a significant survival advantage compared with untreated mice (P ≤ 0.005) or radiation alone (P ≤ 0.003) and an increase in median survival compared with IL-2 alone. Histologically, the combined regimen resulted in a greater degree of tumor destruction, inflammatory response, and vascular damage than that observed with each modality alone. After this combined treatment, no tumor was histologically detected in the para-aortic lymph nodes of these mice, and the lymph nodes were significantly smaller. These findings showed that primary tumor irradiation, either with neutrons or photons, enhanced IL-2 therapeutic effect for the treatment of advanced prostate cancer. This combined modality induced an antitumor response that controlled the growth of prostate tumors and their metastases.

INTRODUCTION

Carcinoma of the prostate is the most common malignant tumor in men, with >180,400 newly diagnosed cases annually, resulting in >31,900 deaths each year (1). Localized prostate carcinoma is sensitive to conventional radiotherapy using megavoltage photons (X-rays); however, residual disease often causes clinical relapse (2). To increase the efficacy of radiation therapy, the use of neutrons was explored. Neutrons are heavy particles produced when a charged particle, such as a deuteron, is accelerated to high energy and then made to impinge on a target such as beryllium (3). The interaction of neutrons with nuclei of atoms of soft tissues sets in motion heavy secondary particles producing dense ionizations more likely to cause critical DNA damage (double strand breaks), which is less repairable than that induced by photons. Neutrons are both more effective in killing hypoxic tumor cells and less dependent on the cell cycle than photons (3). These differences between neutrons and photons result in a greater RBE (3) for neutrons relative to photons and form the radiobiological basis for selecting neutrons for therapy of slow-growing tumors, such as adenocarcinoma of the prostate. In the treatment of locally advanced adenocarcinoma of the prostate, neutrons have been shown to be superior to photons in two randomized multinational Phase III clinical trials (4, 5). At Wayne State University, we have improved the conditions for three-dimensional conformal neutron irradiation using a superconducting cyclotron fully rotational around the patient to produce an isocentric beam operated with a tungsten multirod collimator used to produce irregularly shaped fields (6, 7). Field apertures were designed to conform to the size and shape of the three-dimensional reconstructed tumor volume. After a series of Phase II/III trials for local and locally advanced prostate carcinoma using these techniques, we have established a regimen of neutrons combined with photons that resulted in a significant decrease in tumor recurrence and lower toxicity than that observed in previous trials (8, 9). At 3 years, no evidence of recurrence of disease was found in 91% of patients with stage T1 disease, in 86% of stage T2 disease and 61% of stage T3/T4 disease (9).
Although the local control of stage T3/T4 (61%) was improved over photon radiation alone (35–40%), these findings showed that this treatment alone was insufficient to prevent progression of disease in a large proportion of patients. In patients diagnosed with occult or small clinically palpable lesions, 30–35% develop metastases (10). Once the regional lymph nodes become involved, 85% of patients develop distant metastases in 5 years (10). The predominant site of metastases is osseous in >80% of patients, and only 10–15% of patients present with soft tissue disease (11, 12). Advanced localized or metastatic prostate cancer still presents a difficult therapeutic problem. Metastatic prostate cancer is first treated by androgen blockade; however, within 18 months, it becomes hormone refractory, and patients have an expected survival of <1 year (11, 13). Current research efforts are now devoted to develop alternative therapeutic approaches including immunotherapy, which is based on activation of a host immune response against the tumor cells using cytokines or activated immune cells (reviewed in Ref. 13). The goal of this study is to develop a multimodal approach consisting of radiation therapy and cytokine therapy for the treatment of metastatic prostate cancer.

Cytokines are used in immunotherapy to enhance immune mechanisms directed against tumors (14). Cytokines are proteins produced after activation of lymphocytes, macrophages, or other cells. Cytokines can induce a cascade of activation, proliferation, and differentiation of immune cells and mount an antitumor immune response. The cytokine/lymphokine IL-2, produced by activated T lymphocytes, is critical for immune responsiveness and has been extensively used in clinical trials (14, 15).

We have previously established a mouse metastatic prostate tumor model by injection of human prostate carcinoma PC-3 cells in the prostate of immunodeficient BALB/c nude mice that resulted in the formation of a prostate tumor with metastasis to regional para-aortic lymph nodes (16). We found that day 6 prostate tumors were responsive to systemic injections of IL-2 cytokine therapy (16). We have now investigated the combination of local primary tumor irradiation and IL-2 for day 14 advanced metastatic tumors in this preclinical tumor model. We hypothesize that prior irradiation of the primary prostate tumor will enhance the therapeutic effect of subsequent cytokine therapy. We have designed the apparatus for selective irradiation of tumor-bearing prostates for photon irradiation with an X-ray machine and for neutron irradiation with the cyclotron. This study shows that local prostate tumor irradiation, using neutrons or photons, enhances the effect of IL-2 cytokine therapy.

MATERIALS AND METHODS

Tumor Model

The human prostate carcinoma PC-3 tumor cell line was purchased from American Type Culture Collection (Rockville, MD). PC-3 cells were cultured in culture medium consisting of F-12 K nutrient mixture supplemented with 7% heat-inactivated fetal bovine serum (Life Technologies, Inc., Grand Island, NY), 2 mM glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate (Sigma Chemical Co., St. Louis, MO), 10 mM HEPES buffer, 100 units/ml penicillin/streptomycin, 0.5 µg/ml fungicide, 100 units/ml penicillin/streptomycin, and 0.5 µg/ml gentamicin. As described previously, PC-3 cells were cultured in vitro and then implanted in the prostate of male BALB/c nu/nu nude mice (16). New PC-3 prostate tumor cell lines were generated from prostate tumors (PC-3/PI) or para-aortic lymph node metastases (PC-3/PI-LN) that were more tumorigenic and induced metastatic prostate tumors with faster kinetics than the original PC-3 cells (16). These new PC-3/PI cell lines were used for prostate implantation. Tumor cells were washed twice in HBSS, and a concentration of 4–5 × 10^5 cells in 20 µl of HBSS was injected into the prostates of 4–6-week-old male BALB/c nu/nu nude mice (purchased from Harlan Sprague Dawley Inc, Indianapolis, IN). The prostates of anesthetized mice were exposed through a midline laparotomy incision and by retraction of the bladder and male sex accessory glands anteriorly. Injection of cells was performed with a 27-gauge needle inserted in the prostatic lobe located at the base of the seminal vesicles, as described previously (16). The abdominal wound was sutured using a 4.0 chromic gut suture in a running fashion. Mice were housed and handled under sterile conditions in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. The animal protocol was approved by Wayne State University Animal Investigation Committee.

Prostate Tumor Irradiation

Photon Irradiation. Acrylic jigs were designed to place anesthetized mice in the supine position with their fore and hind limbs restrained by posts to help localize the prostate between the hind legs (Fig. 1, A and B). The prostate position was determined by clamping of the prostate with metallic clips in a surgical procedure, and mice were allowed to recover for 24 h. Then, mice were immobilized in the jigs in a reproducible position, and localization of the prostate was assessed by X-ray radiographs (Fig. 1A). On the basis of these radiographs, lead shields were designed to expose the area of the prostate to photon irradiation while shielding the rest of the mouse body (Fig. 1C). Three jigs were positioned on an aluminum frame mounted on the X-ray machine to irradiate three mice at a time (Fig. 1, B–D). A 6.4-mm lead shield was positioned above the jigs with three cut-outs to allow for irradiation of the prostate (Fig. 1C), as confirmed by double exposure X-ray radiographs (Fig. 1A). The radiation dose to the prostate and the scattered dose to areas of the mouse outside of the radiation field were carefully monitored. Photon irradiation was performed with a Siemens Stabilipan X-ray set (Siemens Medical Systems, Inc.) operated at 250 kV, 15 mA with 1-mm copper filtration at a distance of 47.5 cm from the target.

Neutron Irradiation. The jigs used for photon irradiation were also used for neutron irradiation with the cyclotron; however, a different base plate to hold three jigs was built to fit the cyclotron (Fig. 2, A and B). Tungsten rods, used as a collimator to shield from neutrons, were pushed into the radiation field using a polystyrene foam that was cut to reproduce three neutron fields at the position of the prostates of the three mice, as shown by three hollow cuts in the series of tungsten rods (Fig. 2, C and D). The radiation dose to the prostate and the scattered dose to areas of the mouse outside of the radiation field were carefully monitored. Neutron irradiation was performed in a superconducting cyclotron in which neutrons were produced...
Radiosensitivity of PC-3 Prostate Tumors. After prostate implantation of PC3/PI cells, prostate tumors of ~0.4 cm in size were already observed by days 14–16 (16). This time point was selected for prostate tumor irradiation. On day 16, established PC-3 prostate tumors were selectively irradiated with photons or with neutrons.
various doses of photons (100–800 cGy) or neutrons (50–400 cGy) to compare their radiosensitivity to either form of radiation. Two weeks later, on day 30, mice were sacrificed to assess prostate tumor size. A dose-dependent inhibition of prostate tumor growth was caused by radiation either with photons or neutrons (Fig. 3). Variations were observed in tumor growth of prostate tumors irradiated with low doses of 150–300 cGy photons or 50–150 cGy neutrons because of a mixed response as described below. The decrease in tumor growth was significant starting at doses of 300 cGy photons and 150 cGy neutrons ($P < 0.05$). At higher doses of radiation of 500–800 cGy photons and 250–400 cGy neutrons, the response to radiation was more significant and more consistently observed ($P < 0.01$; Fig. 3). Comparison between the prostate tumor response to photons and neutrons showed that low doses of neutrons were more effective than photons at the same doses (Fig. 3). The RBE...
Radiation and IL-2 for Prostate Tumors

**Figure 4** PC-3 prostate tumor response to radiation combined with systemic IL-2 therapy. Day 14-established prostate tumors were irradiated with either 500 cGy photons or 250 cGy neutrons. One day later, IL-2 therapy was initiated at 30,000 CU per day for 4 days. Two additional weekly cycles of 4 days of the same IL-2 dose were given. After completion of IL-2 therapy on day 30, the mice were sacrificed, and the prostates, including the tumors, were measured in three dimensions. Two separate experiments are shown with photon radiation and neutron radiation. To compare between them, the percentage of tumor inhibition is reported; bars, SE. The percentage of inhibition is based on mean tumor volume of five to six mice and was calculated relative to the mean size of untreated tumors.

Calculated as the ratio of photon dose to neutron dose causing 50% inhibition was found to be 2 for prostate tumors when estimated based on the tumor volume and the tumor weight from the graphs depicted in Fig. 3. A good correlation was observed between tumor volume and tumor weight.

Pathological observations showed that untreated mice had large vascularized prostate tumors of 0.8–1.5 cm in diameter compared with the size of normal prostate of 0.3 cm. After photon radiation at doses of 500–800 cGy or neutron radiation at 250–533 cGy, some of the mice showed almost no sign of tumor in prostate, whereas others had remaining white, hard nodules of 0.4–0.7 cm that seemed nonvascularized. At lower doses of 300 cGy photons or 150 cGy neutrons, a mixed response was observed with large tumors (0.9–1.0 cm) and small tumors (0.5 cm). When the effects of neutrons and photons are compared, these gross pathological observations indicate an RBE of at least 2 that correlates with the RBE calculated from the graphs in Fig. 3.

On the basis of previous studies, measurement of para-aortic lymph nodes in this tumor model can be used as an indicator of tumor metastasis and invasion into the lymph nodes. Large prostate tumors are always accompanied by enlarged para-aortic lymph nodes from which tumor cells can be isolated (16). The para-aortic lymph nodes are located in the lumbar part of the spine along the aorta; we estimate that some of them are in the field of radiation in the proximity of the prostate whereas others are located higher along the lumbar spine above the field of radiation. In contrast to a size of 0.1–0.15 cm in normal mice, large para-aortic lymph nodes of 0.3–0.5 cm were prominent in untreated mice bearing large prostate tumors and were located both in the radiation field and outside of the radiation field. After prostate tumor irradiation, the lymph nodes located in the radiation field received the same dose of radiation than the prostate whereas the ones located above the radiation field were shielded. Scattered radiation outside of the radiation field was carefully monitored and found to be negligible, ~1–2% of the treatment dose. In mice treated with doses of 500–800 cGy photons or 250–533 cGy neutrons most para-aortic lymph nodes were not as enlarged as described below.

**Combination of Radiation with Cytokine Therapy.** As shown in Fig. 3, the tumor inhibition obtained with 500 cGy photons or 250 cGy neutrons was still significant (>75%). Therefore, these intermediate doses were combined with systemic injections of IL-2, and the therapeutic effect was monitored on the tumor size growth. Established PC-3 advanced prostate tumors were selectively irradiated on day 14 with 500 cGy photons or 250 cGy neutrons. One day later, systemic IL-2 therapy was initiated and administered in 3 weekly cycles of 4 days i.p. injections of nontoxic doses of IL-2 at 30,000 CU/day. Mice were sacrificed at the end of the treatment by day 30 after cell injection to assess primary tumor size and metastases. IL-2 therapy alone induced a decrease of 53% in prostate tumor size, but variations were observed between mice (P > 0.05). Photon radiation alone caused a 77% decrease in tumor size (P < 0.05) compared with untreated mice (Fig. 4). A trend in further tumor size reduction to 94% was observed with the combination of both therapies that was highly significant compared with untreated mice (P < 0.01) but not to each therapy alone (Fig. 4). This could be attributable to the fact that the tumor nodules are still present and measurable after therapy, but they do contain significant areas of necrosis and fibrosis as clarified by histology studies below (Table 1). The tumor reduction obtained with the combined therapy was consistently observed after tumor measurement of three dimensions (Fig. 4) and by weight (data not shown). In all of the mice tested, increase in the size of para-aortic lymph nodes correlated with increased primary tumor size. Therefore, the response of this advanced prostate tumor model to each modality alone or both combined could also be monitored by the size of the regional para-aortic lymph nodes and followed a striking pattern. Enlarged lymph nodes were observed in 100% of untreated mice compared with 60, 50, and 0% in IL-2, radiation, and radiation + IL-2-treated mice.

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**Table 1** Summary of histological observations

<table>
<thead>
<tr>
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<th>Percentage of surviving tumor cells</th>
<th>Inflammatory infiltrates</th>
<th>Fibrosis</th>
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<tr>
<td>Control</td>
<td>&gt;95</td>
<td>±</td>
<td>−</td>
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<tr>
<td>IL-2</td>
<td>60–70</td>
<td>+++</td>
<td>−±</td>
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<tr>
<td>Photon radiation</td>
<td>30–40</td>
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<td>Neutron radiation</td>
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<tr>
<td>Photon + IL-2</td>
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Quantitation of the histological findings described in Fig. 6 is shown. The degree of inflammatory infiltrates and fibrosis was scaled from mild (+) to heavy (+++).
The mean lymph node size in the control group was 14 ± 5.1 mm³ (volume ± SE) and was decreased to 11 ± 4.7 mm³ with IL-2 alone and to 4.6 ± 1.2 mm³ with radiation alone. After the combined treatment, the lymph nodes were 1.3 ± 0.3 mm³, thus significantly smaller than those after no treatment, IL-2, or radiation alone (P < 0.05). Therefore, the combination of photon radiation with IL-2 therapy resulted in a greater control of primary prostate tumor growth and metastasis to regional lymph nodes.

In a separate experiment, the inhibition in tumor growth induced by 250 cGy neutrons (>75%) was comparable with that induced by 500 cGy photons and was further increased by addition of IL-2, as found for photons combined with IL-2 (Fig. 4). The primary tumor growth inhibition mediated by neutron radiation alone or combined with IL-2 was highly significant compared with untreated mice (P < 0.01) and was consistently observed in all mice. The effect on tumor metastasis to lymph nodes induced by neutrons and IL-2 also followed a comparable pattern than that observed with photons and IL-2 therapy, resulting in small lymph nodes.

After tumor radiation combined with IL-2 therapy, most of the mice had small hard nodules in the prostate that grossly had the appearance of scar tissue rather than tumor. To test whether these remaining nodules could cause tumor recurrence and to assess the long-term effects of the combined therapy, survival studies were performed. Mice received a comparable treatment to that described above with prostate radiation at intermediate doses of 500 cGy photons or 250 cGy neutrons, but a higher optimal dose of IL-2 was given in the first weekly cycle to augment the effect of IL-2 therapy. After radiation, mice were treated for the first 4 days with an optimal dose of 40,000 CUs/day and then received 30,000 CUs/day for 5 days per week for 2 additional weeks. We have found previously that this IL-2 regimen is tolerated by the nude mice, whereas doses higher than 40,000 CUs/day will result in severe toxicity and death. The various treatments of photon or neutron radiation alone, IL-2 alone, and both combined were all tested in the same experiment to compare between each modality alone and the combined modalities. Mice were monitored daily for survival, and moribund animals were sacrificed and autopsied. On day 63, all remaining mice were sacrificed. The median survival of prostate tumor-bearing mice in the control untreated group was 45 days and was increased to 59 days by photon radiation and IL-2 and to 63 days (or higher) by neutron radiation and IL-2 (Fig. 5). Comparisons between the survival curves of treated mice and untreated mice and between the various treatments were made using the log-rank test. Compared with untreated mice, prostate tumor irradiation alone with intermediate doses of either 500 cGy photons or 250 cGy neutrons did not increase mouse survival significantly (P > 0.3). IL-2 alone, at optimal doses, seemed to increase mouse survival, but it was not significant (P = 0.07). In contrast, the combination of IL-2 with photon radiation (P = 0.005) or neutron radiation (P = 0.001) significantly increased mouse survival compared with untreated mice (Fig. 5). Comparisons between one-modality treatment and two-modality treatment showed that the combination of radiation and IL-2 caused a significant survival advantage compared with photon or neutron radiation alone (P ≤ 0.003) and an increase in median survival compared with IL-2 alone. Autopsy of moribund mice during this experiment showed that untreated mice had large prostate tumors with metastasis to lymph nodes, occasionally to seminal vesicles and mesentery. The majority of the mice treated with radiation alone or IL-2 or both showed tumor recurrence.

In the same experiment, to control for radiation-induced toxicity to normal organs, mice not transplanted with PC-3 cells in the prostate were irradiated with 500 cGy photons and 250 cGy neutrons in the prostate area using the apparatus described in Figs. 1 and 2. No reduction in mouse weight or size or signs of disease were observed over 45 days after radiation, and the mice appeared healthy. At autopsy at this time point, the size and appearance of the prostate, gonads, seminal vesicles, and bowels were normal. Thus, local radiation in the prostate area with 500 cGy photons and 250 cGy neutrons does not cause toxicity to normal tissues.

**Histology of Prostate Tumors Treated with Radiation and IL-2.** To investigate *in situ* the effect of radiation or immunotherapy or both on prostate tumors, the histology of prostate tumors treated with radiation, IL-2, or both was evaluated in a separate experiment. Mice were treated with 800 cGy photons or 533 cGy neutrons delivered to tumor-bearing prostates on day 16 and then were administered IL-2 similarly to the

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**Fig. 5** Survival of PC-3 prostate tumor-bearing mice treated with prostate tumor radiation combined with systemic IL-2 therapy. Day 14-established PC-3 prostate tumors were irradiated with 500 cGy photons or 250 cGy neutrons. One day after radiation, mice were treated with injections of IL-2 at 40,000 CUs/day for 4 days and then received two weekly cycles of 5 days of IL-2 at 30,000 CUs/day. Twelve mice/group were used and followed for survival.
experiments described in Fig. 4. On day 31, mice were sacrificed, and prostate tumors and regional para-aortic lymph nodes were resected and processed for histology. Histologically, untreated PC-3 prostate tumors presented as a poorly differentiated epithelial neoplasm consisting of pleomorphic tumor cells with large hyperchromatic nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm (Fig. 6A). These tumors had a myxoid background and loosely cohesive growth pattern. There was no significant tumor necrosis or associated inflammatory infiltrates (Table 1). After IL-2 therapy, tumors showed patchy necrosis, clusters of lymphocytes, and PMN cells (Fig. 6B; Table 1) as well as vascular damage manifested by multifocal hemorrhages (Fig. 6C). After high doses of radiation alone or combined with IL-2, tumors grossly presented as small hard nodules, which histologically contained large areas of necrosis and fibrosis (Table 1). After photon or neutron radiation alone, there was a significant decrease in viable tumor cells that was associated with prominent infiltrates of PMN and lymphocytes as well as fibrosis (Table 1; Fig. 6, D and G). The combination of photon or neutron radiation induced a much greater reduction in viable tumor cells associated with more extensive tumor apoptosis, inflammatory infiltrates, and hemorrhages (Fig. 6, E, F, H, and I) than that seen with radiation or IL-2 therapy alone (Table 1). Only rare viable tumor cells were detected focally.

The response of metastases to therapy was also confirmed by histology studies of para-aortic lymph nodes. On day 31, a heavy infiltration of tumor cells was observed in para-aortic lymph nodes of untreated mice with ~50% of the lymph node
cells replaced by tumor cells (Fig. 7A). These observations correlate with our previous findings showing that enlarged lymph nodes are attributable to tumor metastasis from large primary tumors (16). In contrast, the para-aortic lymph nodes of mice treated with either photon or neutron radiation and IL-2 were small and histologically did not show tumor infiltration (Fig. 7B).

DISCUSSION

To improve the control of local prostate tumor growth and metastases, the goal of our studies was to test the combination of photon or neutron irradiation of the primary prostate tumor with systemic cytokine therapy in a preclinical metastatic model of prostate carcinoma. The rationale for combining these modalities was to reduce large tumor burdens localized in the prostate by radiation and eradicate residual tumor and metastases by inducing an antitumor immune response with the cytokine therapy.

We have shown previously that IL-2 can induce an antitumor response in the two major sites of prostate carcinoma, i.e., prostate and bone using two animal models of human PC-3 cells injected either in the prostate or the femur of nude mice, resulting in a metastatic prostate tumor model and a bone tumor model, respectively (16, 17). This effect was observed after systemic IL-2 treatment of established tumors on days 5 or 6 after PC-3 cell injection, at an early stage of tumor development. A significant inhibition in the growth of prostate and bone tumors was observed associated with IL-2-induced tumor destruction, vascular damage, and inflammatory infiltrates of PMN cells and lymphocytes (16, 17). These observations in tumor models, coupled with prostate cancer radiosensitivity in patients, suggest a potential for combining irradiation with immunotherapy for the treatment of metastatic prostate carcinoma.

In addition to conventional photon radiation for prostate cancer, we have also tested neutron radiation, a more effective form of radiation, because of the unique cyclotron facilities and the expertise we have developed in our institution for the use of neutrons in cancer patient therapy (6–9). To test the multimodality approach of radiation followed by cytokine, we have used in this study the PC-3/nude mouse prostate tumor model at a more advanced stage of disease, on days 14–16 after PC-3 cell injection, when the prostate tumor was already ~0.4 cm. After development of the appropriate radiation apparatus for selective prostate tumor radiation with photons and neutrons, we have demonstrated that PC-3 prostate tumors in nude mice are sensitive to radiation in a dose-dependent manner. Similar to observations of greater efficacy of neutrons compared with photons in patients (4, 5, 8, 9), we found that neutrons were more effective than photons for the inhibition of PC-3 prostate tumors in mice and could be used at lower doses than photons with an RBE of 2. At doses of neutrons (250–533 cGy) and photons (500–800 cGy), a significant inhibition of tumor growth (>75%) was observed, resulting in small, hard tumor nodules.

When intermediate doses of neutrons (250 cGy) or photons (500 cGy) were administered to established prostate tumors, followed by three weekly cycles of IL-2 therapy, the tumor inhibition was further increased to 90% and higher. This effect was very consistently observed, with most of the mice showing remaining small hard nodules in the prostate, which had more the appearance of scar tissue than that of a tumor. Furthermore, the size of the regional para-aortic lymph nodes of mice treated with the combined therapy was significantly smaller than those of untreated mice or mice treated with a single modality. These data indicate a good correlation between tumor size and lymph node enlargement associated with metastasis as shown previously (16). Therefore, the combination of photon radiation with IL-2 therapy resulted in a greater control of primary prostate tumor growth and inhibition of tumor metastasis to regional lymph nodes than each modality alone. Survival studies performed over 2 months with the same conditions of prostate tumor irradiation and IL-2 therapy showed that prostate tumor radiation alone or IL-2 therapy alone did not significantly increase mouse survival. The combination of radiation and IL-2, however, caused a significant survival advantage compared with untreated mice ($P \leq 0.005$) or photon or neutron radiation alone ($P \leq 0.003$) and an increase in median survival compared with IL-2 alone. Autopsy of moribund mice during this experiment showed that the majority of the mice treated with radiation alone or IL-2 or both had tumor recurrence. Toxicity to normal tissues was not seen after photon or neutron radiation of the prostate area in normal mice not bearing prostate tumors.

Histologically, either radiation or IL-2 induced tumor destruction and inflammatory infiltrates. IL-2 caused a more intense infiltration in PMN cells and lymphocytes as well as vascular damage as found previously (16). After photon or neutron irradiation, in addition to areas of tumor destruction and inflammatory infiltrates, areas of fibrosis were seen probably as a reaction to tumor destruction. The combined regime resulted in a greater tumor destruction, inflammatory infiltrate, and vascular damage that seemed to be more pronounced with neutrons than photons. This effect was visualized as a marked decrease in tumor cellularity because of intense tumor cell loss, with very
loose areas containing few tumor cells and numerous apoptotic cells. Clusters of inflammatory cells were often seen around tumor cells. These histological findings are indicative of the in situ effect of the combined therapy on the primary prostate tumor and corroborate our gross pathological observations of remaining small hard nodules, which resembled scar tissue rather than tumor. After the combined therapy, no tumor was histologically detected in the para-aortic lymph nodes in correlation with the small lymph node size observed in these mice and in contrast to invasion of tumor in the enlarged lymph nodes from untreated control mice. These findings showed that primary tumor irradiation, either with neutrons or photons, enhanced IL-2 therapeutic effect for the treatment of advanced metastatic prostate cancer.

These studies in prostate carcinoma confirm our previous findings in a renal carcinoma tumor model of an enhancement of therapeutic effect by combination of local tumor irradiation with IL-2 therapy compared with each modality alone (18, 19). The histological observations of tumor cell destruction, vascular damage, and inflammatory infiltrates were also comparable with those seen in the prostate tumor model (18, 19). It is interesting to note that the effects of the combined therapy observed in the renal carcinoma syngeneic model in normal mice were reproduced in the prostate PC-3 tumor model in immunodeficient nude mice, including the massive tumor infiltration by inflammatory cells. Although T-cell function is impaired in nude mice, NK cell, macrophage, and PMN activities are functional and can be enhanced by cytokines. Enhanced NK activity by cytokines is associated with the generation of lymphokine-activated killer cells that are capable of killing a larger spectrum of tumor cells than NK cells (14). Activation of macrophages by cytokines induces tumoricidal activity (14). These two types of killer cells may be sufficient to lead to a significant nonspecific antitumor immune response as observed in the present study. Our findings, both in the prostate tumor model and the renal carcinoma model, suggest that radiation therapy causes changes in the tumor cells and the tumor environment, which increase the tumor susceptibility to destruction by the immune system activated by IL-2.

In the advanced metastatic prostate tumor model, the combined radiation/IL-2 regimen induced an antitumor response that controlled the growth of prostate tumors and their metastases. However, this was not a curative protocol; as demonstrated in survival studies, mice died of recurrent tumor. Further studies are under way to combine this multimodality approach of tumor irradiation and nonspecific IL-2 cytokine therapy with a more potent and specific inducer of antitumor immune response, such as a prostate cancer vaccine in a syngeneic prostate tumor model (20, 21).

REFERENCES

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