Effects of IL-3 Gene Expression on Tumor Response to Irradiation in Vitro and in Vivo

Chi-Shiun Chiang, Randi G. Syljuåsen, Ji-Hong Hong, Anne Wallis, Graeme J. Dougherty, and William H. McBride

Department of Nuclear Science, Tsing-Hua University, Hsin-Chu 30043, Taiwan [C.-S. C.]; Department of Radiation Oncology, University of California, Los Angeles, Los Angeles, California 90095 [R. G. S., G. J. D., W. H. M.]; Department of Radiation Oncology, Chang Gung Memorial Hospital, Tao-Yuan 33333, Taiwan [J.-H. H.]; and Terry Fox Laboratories, Vancouver, British Columbia, Canada [A. W.]

Abstract

Expression of a murine interleukin 3 gene in murine fibrosarcoma cells (FSA-JmIL-3) did not alter their survival after in vitro irradiation. However, FSA-JmIL-3 tumors established in vivo were much more sensitive to irradiation than was the parental tumor. Following 25 Gy of irradiation, parental fibrosarcoma tumors regrew after a growth delay of 10 days, but FSA-JmIL-3 tumors continued to regress. Examination of the cellular composition of tumors following irradiation revealed that, instead of tumor cell repopulation, the FSA-JmIL-3 tumors became heavily infiltrated with lymphocytes, indicating that the effect of irradiation was to allow the IL-3–elicited cellular immune response to infiltrate the tumors and mediate rejection. This study indicates that combining gene immunotherapy approaches with radiotherapy might increase the effectiveness of both, and it seems logical to pursue such treatment options.

Introduction

For more than 2 decades, it has been recognized that multimodality treatment of cancer is in many cases better than any single modality. For example, radiation therapy as a single modality may be effective at achieving local tumor control, but metastases frequently develop outside the radiation field. The concept of combining radiation therapy with other systemic treatments is therefore appealing. For those tumors for which it is difficult to gain local control using radiotherapy, radiosensitization might be achieved by altering the intrinsic radiosensitivity of the tumor or the in vivo tumor microenvironment. The optimal strategy might, however, translate tumor cell kill into the generation of a state of systemic antitumor immunity to cope with both local residual tumor deposits and systemic micrometastatic disease.

Recent studies have indicated the feasibility of using gene therapy approaches to enhance the probability of achieving local tumor control after radiotherapy (1, 2). In vivo replacement of the tumor suppressor gene p53 increases the sensitivity of a tumor to irradiation (3, 4), as do certain prodrug strategies such as the use of HSV-Tk gene transfer along with nucleoside analogues (5). We have shown in previous studies that expression of certain cytokine genes, such as IL-6 or IFN-α, can enhance intrinsic radiosensitivity of tumor cells in vitro (6), and when expressed intratumorally, cytokines can cause an influx of host cells into tumors with microenvironmental changes that could impact the response to radiotherapy in a number of ways (7, 8).

In addition to possibly altering tumor responses to irradiation, tumor-directed cytokine gene expression has been shown in many animal tumor models to reduce tumorigenicity and enhance tumor immunogenicity. Vaccines from such genetically modified tumor cells in some cases can cure small, established parental tumors and prevent the development of distant metastasis (9–12). The effects vary considerably from tumor model to tumor model, with the cytokine being used, with the level of expression, and depending on many other variables. This makes it difficult to determine the best cytokine gene to use in vaccine-based gene therapy strategies, but numerous clinical trials have been initiated based on preclinical data.

The results of these clinical trials with vaccine-based tumor-directed cytokine gene therapy have to date proven somewhat disappointing (13). This may be because of “small-scale clinical trials” or of a lack of “sufficient controls to evaluate the true merits of gene therapy” (13). Alternatively, the apparent “failure” of vaccine-based approaches may be due simply to a failure to generate sufficient immunity to deal with growing solid tumor masses. This may be why tumors producing cytokines such as IL-7 (8) and IL-3 (7, 14) will still grow if sufficient cells are injected, although factors other than tumor burden may be important. Such factors include tumor-associated immunosuppression, lack of tumor-associated antigens, and failure of lymphocytes to traffic to tumor sites. In any event, the implication is that immunotherapy on its own may prove to be relatively ineffective against clinically detectable tumors.

This study was initiated in an attempt to prove the principle that a combination of cytokine gene therapy and radiotherapy may be an efficient way to enhance the efficacy of both. We used IL-3–transfected tumors as a model. Such tumors are more sensitive than parental tumors to irradiation in vivo but not in vitro, and postradiotherapy they develop a dramatic T-cell infiltrate and a high level of systemic immunity.

Materials and Methods

Mice. Inbred female C3H/Kam//Sed mice maintained in the specific pathogen-free mouse colony of the Department of Radiation Oncology, University of California, Los Angeles, were used. They were 12 weeks old at the beginning of the experiments.

Tumors and Tumor Irradiation. A moderately immunogenic methylcholanthrene-induced fibrosarcoma (PSA) that is syngeneic to C3H/Kam//Sed was used. Tumors were generated by inoculating 5 × 10⁵ viable PSA cells into the right thighs of mice. The different number was to compensate for the reduced tumorigenicity of the FSA-JmIL-3 tumor cells and allow tumors to develop synchronously. Tumors 6–8 mm in diameter were irradiated using a 250-kVp Phillips’ X-ray source (dose rate, 1.2 Gy/min) with the rest of the body shielded to a single dose of 25 Gy. This would be a relatively high dose if used clinically, and more often fractionated rather than single doses would be given, but the purpose here was simply to demonstrate proof of a principle. Tumor growth was determined by measuring three mutually orthogonal tumor diameters at 2–3-day intervals with a Vernier caliper and calculating the mean values. This measurement was...
performed until tumors reached 12 mm in diameter, when mice were euthanized. For in vitro irradiation, cells were irradiated in a Gammacell 220 (Atomic Energy Ltd., Toronto, Ontario, Canada) with a cobalt source at a dose rate of 3.3 Gy/min. After irradiation, cells were plated in 100-mm diameter Petri dishes, and colonies of greater than 50 cells were counted on day 12 after staining by Giemsa.

Gene Transduction. The Moloney murine leukemia virus-based vector Jzen.1 was used to introduce and express the full-length cDNA for mIL-3 into cultured tumor cells, as described previously (7). IL-3 gene expression was examined by RPA analysis, and protein production was examined by survival of the IL-3-dependent cell line DOCAP. All of the experiments in this study have also been performed using a control cell line transduced by the same vector containing only a neomycin resistance gene. Because the results were essentially identical to those obtained with the parental line, the data are omitted for the sake of clarity.

RPA. The total RNA was isolated from tumors using single-step acid guanidinium thiocyanate-phenol-chloroform extraction. Expression of ILs (IL-1α/β, IL-2, IL-3, IL-4, IL-5, and IL-6), IFN (IFN-γ), TNFs (TNF-α/β), TNF-R-p55 and -p75, IL-1R-p60 and -p80, IFN-γR, and IL-6R was measured by RPA (15). The templates for making these probes were provided kindly by Drs. M. V. Hobbs and I. L. Campbell (Scripps Research Institute, San Diego, CA). RPA was performed as described previously (15).

Characterization of Tumor-infiltrating Host Cells. The host cell populations present within dispase-elicited single-cell digests of normal and genetically modified tumors were identified by flow cytometry using a FACScan machine (Becton Dickinson, Mountain View, CA) as described previously (7). Host and tumor cell populations were gated using forward and side scatter profiles.

Results

Effects of IL-3 Gene Transduction on Tumor Cells. The transduction of FSA tumor cells with the mIL-3 gene induces increased expression of MHC class I, CD44, and intercellular adhesion molecule 1 molecules, but not MHC class II (7), indicating that IL-3 has autocrine effects on these cells. In this study, we further examined the effects of IL-3 on the expression of several cytokine and cytokine receptor mRNAs by RPA. FSA does not express the proinflammatory cytokines TNF-α/β, IL-1α/β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, or IFN-γ (Fig. 1A), and this did not change following mIL-3 gene transduction. IL-3, at a high level, was the only cytokine detected in FSA-JmIL-3 tumors. FSA tumors did, however, express significant levels of IL-1Rp80/60, TNF-Rp75/55, IL-6R, and IFN-γR, and after
IL-3 GENE RADIOTHERAPY OF CANCER

Effects of IL-3 Gene Expression on Tumor Response to Irradiation. We had shown previously that IL-3-transduced tumor cells were more immunogenic than parental cells as shown by their ability as irradiated vaccines to protect mice against the growth of viable parental cells. Also, they had decreased tumorigenicity. This increased immunogenicity and decreased tumorigenicity could however be overcome by increasing the number of tumor cells injected (7, 14), and FSA-JmIL-3 tumors would grow progressively although at a slightly slower rate.

In this study, we examined the effects of irradiating IL-3 gene-transduced tumors with the aim of allowing IL-3-enhanced tumor immunity to better express itself in the form of tumor regression. An advantage of radiation treatment is that radiation can be delivered directly to the tumor without compromising host systemic immunity. A single dose of 25 Gy of radiation caused parental FSA tumors to regress only temporarily, and they regrew after 10 days (Fig. 2). This was expected, because the TCD_{50} for 6 mm-diameter FSA tumors is 37 Gy (16). In contrast, FSA-JmIL-3 tumors continued to regress after irradiation. In almost all cases, tumors disappeared completely, and mice were tumor free for at least 3 months. At the same time, these mice developed long-term specific immunity to parental tumor. They were completely resistant to the s.c. challenge of 5 X 10^6 of FSA tumor cells given 3 months later (data not shown).

Effects of IL-3 Gene Expression on the Intrinsic Radiosensitivity. One possible reason for the increased radiation response of FSA-JmIL-3 in vivo could have been an alteration in intrinsic radiosensitivity of the tumor as a result of the autocrine action of IL-3. A clonogenic survival assay was performed to examine this possibility.

The data show that the FSA-JmIL-3 cells have an almost identical survival curve to that of parental FSA tumors in response to various radiation doses (Fig. 3). A similar result was found when the survival of FSA and FSA-JmIL-3 tumor cells, taken 1 day after in vivo irradiation, were compared in an in vitro clonogenic assay (data not shown). Differences in the intrinsic radiosensitivity of FSA and FSA-JmIL-3 tumors cannot therefore be the explanation for the observed in vivo radiosensitivity of FSA-JmIL-3 tumors.

Host Cellular Response within Tumors following Irradiation. To examine the role of host immunity in the irradiation response of FSA-JmIL-3 tumors, their cellular composition at various times after 25 Gy irradiation was followed by flow cytometry using antibodies against cell surface markers including Mac-1 and F4/80 for macrophages, CD4 and CD8 for T lymphocytes, and B-220 for B cells. Size and granularity were also used to identify the various cellular populations. In unirradiated FSA-JmIL-3 tumors, the major change from the parental type is a higher composition granulocytes (7). Lymphocyte numbers are increased slightly, but the percentages of macrophages are similar to FSA.

After 25 Gy of irradiation, in FSA tumors, the number of tumor cells decreased temporarily but increased subsequently consistent with repopulation and tumor regrowth (Fig. 2). Over the initial 2-week postirradiation period, macrophages within the FSA tumor were increased proportionately and lymphocytes decreased, consistent with the known relative radiosensitivities of these cell types. By day 19, the balance between host and tumor cells was re-established to what it was prior to irradiation (Fig. 4A).

In IL-3-expressing tumors, lymphocyte numbers decreased after irradiation as they did in the parental tumors, but at 7 days they reappeared and were obvious on forward and side scatter profiles within FSA-JmIL-3 but not FSA tumors. Thereafter, in contrast to FSA tumors, irradiated FSA-JmIL-3 tumors became massively infiltrated with lymphocytes. At the same time, the tumor cell number decreased continuously until the tumors became too small to analyze (Fig. 4B). Most of the infiltrating lymphocytes were CD8^+ T cells, but CD4^+ cells were also well represented. This influx of immune cells...
was consistent with the continued regression of the FSA-JmIL-3 tumors.

Discussion

This study shows that IL-3 gene insertion and expression potentiated the responses of a murine FSA tumor to ionizing irradiation. This was not due to alteration in intrinsic radiosensitivity of the tumor cells. Rather, taken together with data from ourselves and others that IL-3 enhances host antitumor immunity (7, 14), the fact that the irradiated FSA-JmIL-3 tumors became massively infiltrated with lymphocytes after irradiation and the fact that the mice developed a state of long-lasting tumor-specific immunity, the data suggest that the irradiation allowed expression of IL-3-enhanced tumor immunity, and this in cooperation with the cytotoxic action of radiation mediated tumor regression.

The mechanism by which IL-3 enhances tumor immunogenicity may be complex. Most likely it exerts its effects through activation of antigen-presenting cells (APCs). IL-3 can expand a subset of macrophages (14) that are particularly potent APCs with an increase in expression of coaccessory molecules, including MHC class II molecules and IL-1 (17). A recent paper has also shown that IL-3, like granulocyte macrophage colony-stimulating factor, can cooperate with TNF-α to induce the growth of dendritic cells, which are also particularly potent APCs (18). An alternative mechanism, which does not exclude the preceding, is that IL-3 gene expression up-regulates the expression of MHC class I molecules and other cell adhesion molecules on the tumor, and these may play a role as accessory factors in immune stimulation. These autocrine effects of IL-3 may also enhance the ability of the FSA-JmIL-3 tumor cells to be lysed by cytotoxic lymphocytes. In addition, the IL-3-induced up-regulation of TNF-R expression described in this study could be a factor, because the FSA-JmIL-3 tumor cells are more susceptible to lysis by TNF-α than are cells of the parental line.4

Potential synergy between radiotherapy and immunotherapy has been the subject of surprisingly few clinical or experimental studies. Whole-body irradiation is known to eliminate radiosensitive suppressor cells, and in animal models this can lead to tumor regression. Local irradiation may, however, act simply by decreasing the tumor burden and arresting tumor cell proliferation, allowing the immune system to be more effective. This was the explanation given for the
success of adoptive immunotherapy with tumor-infiltrating lymphocytes and IL-2 in combination with irradiation against established 7-day hepatic metastases (19). Although this may in part explain the increased radiosensitivity of the FSA-JmIL-3 tumors, other mechanisms are probably also operating. FSA-JmIL-3 tumors became massively infiltrated with lymphocytes 7 days after irradiation. This did not happen in parental tumors, although the FSA is moderately immunogenic. IL-3 may therefore assist lymphocyte recruitment into the irradiated tumor site. Alternatively, IL-3, through its effects on APCs, may inhibit the generation of systemic tumor-associated immune suppression, allowing the maintenance of better expression of enhanced tumor-specific immunity.

This study raises issues as to how best to combine immunotherapy with other conventional cancer therapies. Immunotherapy has had the occasional fascinating successes in individual patients but has often failed in full clinical trials. Using localized radiotherapy that has little effect on systemic immunity would seem one possible strategy that might increase its success rate. On the other hand, this may not be sufficient to achieve local tumor regression and elimination of metastatic deposits. Developing strategies that allow the tumor cytotoxicity from radiotherapy to be translated directly into the generation of systemic immunity may be required. Whether in situ intratumoral manipulations to increase tumor immunogenicity will be necessary or whether this goal can be gained through the use of vaccines administered at sites distant from the tumor will have to be determined. In any event, the option of combining gene immunotherapy with radiotherapy to improve tumor control probability seems worthy of further development.

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References


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