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KINETIC AND BIOCHEMICAL STUDIES ON TUMOR GROWTH

Final Report

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ABSTRACT

The normal growth kinetics and the recovery kinetics following therapeutic perturbations with drugs and x-irradiation for the mouse duodenal crypt cells and the S102F and Slow line tumor cells have been studied in depth. The rationale being that a detailed understanding of these phenomena would enhance our knowledge about tumor biology *per se* and also expand our appreciation of how normal and malignant tissues respond to and recover from therapeutic insults. Hopefully, such knowledge will eventually allow one to design logical, "optimal", protocols for cancer therapy.

During the course of this contract, a new direct method (The ^{125}I-IUdR Method) for measuring cell loss from solid tumors *in vivo* was developed. Also it was established that the new flow cytometry methodology would be very useful for studying the normal and perturbed kinetics of solid tumors. The extensive hydroxyurea data as well as the preliminary adriamycin and x-irradiation data which have been collected on the recovery kinetics of the duodenum and mammary tumors have advanced our awareness of the complexity of these phenomena and how different the effects are when different drugs are used either alone or in conjunction with x-irradiation.

INTRODUCTION

This project was initiated with the major objective being a better understanding of the biology of the C3H mouse mammary tumors; specifically, a detailed understanding of the kinetic events at the cellular and subcellular levels which contribute to volumetric growth and/or regression. The rationale being that an understanding of tumorigenesis *per se* requires knowledge of the dynamics of the host-tumor interactions, and such knowledge is also essential for understanding how therapeutic agents such as ionizing irradiation and drugs influence tumor growth and affect cures. Thus, included in the original objective, is a more thorough knowledge of the kinetic response to insults by therapeutic agents and a comparison of this response in tumors to that in the mouse duodenum. A recent expansion of the major objective includes studies of the biochemical alterations concerned with differentiation which may be associated with the neoplastic state in these murine tumors.

This final report will be divided along the lines of the major projects which have been undertaken. For each project, the background will be reviewed briefly, the published work summarized, and the incomplete work-in-progress documented.

1. The ^{125}I-IUdR (5-iodo-2'-deoxyuridine) Method for Measuring Tumor Cell Loss (See Publication #s 1-5, & 10)

The fact that cells are lost from solid tumors has been recognized for many years; however, only in the last decade has cell loss been the subject of extensive quantitative analysis. Now, it is well recognized that the cell-loss rate must be determined in a comprehensive analysis of unperturbed tumor growth as well as therapeutically perturbed growth. Traditionally, cell loss has been estimated by combining autoradiographic and volumetric growth-curve analysis; however, this approach is indirect and very time consuming. Thus, the ^{125}I-IUdR technique was hypothesized as a direct way for measuring cell loss.

The evaluation of the ^{125}I-IUdR method for measuring cell loss from C3H mouse mammary tumors has ben completed (See pub. # 4, and manuscript in prep. #1) and can be summarized as follows: 1) IUdR is reutilized but at a much lower rate than thymidine, 2) The ^{125}I-IUdR method cannot be used for *in situ* tumors because of the high and variable radioactivity in the skin overlying the tumors as well as in the acid-soluble fraction of the tumors, but 3) it can be utilized *in vivo* if one removes the tumors and separates the acid-soluble fraction from the macromolecular fractions. This method will not give absolute rates of cell loss because of the reutilization; however, this relatively simple approach does give results which agree quite well with the laborious and indirect methodology of combined autoradiography and volumetric growth-curve analysis.

During the course of these experiments, we had observed evidence for IUdR toxicity in the mouse duodenum. This toxicity (i.e. both cytotoxicity and unusually low cellular labeling indices) were observed in the crypts at doses of 75 μCi (0.01 - 0.02 μ mole) ^{3}H-IUdR per mouse. In summary (Pub. #10), even relatively low doses of IUdR can cause subtle changes in the cellular kinetics; thus, to avoid problems, the dose should be carefully evaluated for each experimental situation.

2. Hydroxyurea (HU) Effects on Tumor Cell and Duodenal Crypt Cell Kinetics (See Publication #'s 7-9, 11, 14 & 15 and manuscript in Prep. #2)

Tumor biologists have for many years expected that the data derived from the formal cellular kinetic analysis of normal and malignant tissue would lead to logical improvements in protocols for radiation therapy as well as chemotherapy. Unfortunately, direct impact on the clinic has been minimal. The reasons are multiple but one salient fact is that there is very little data on the recovery kinetics of either normal or malignant tissue following a therapeutic insult.

We began using HU because we felt that this drug would be useful for studying the basic mechanisms involving cell-cycle perturbations in an animal-tumor system. Hopefully, these data could be used to design logical protocols for fractionated chemotherapy and combined modality therapy. HU was chosen because its effects on cells had been studied extensively in vitro, and the major effects which are of interest to use (i.e. S-phase cytotoxicity, a G_1/S reversible block, and partial synchronization of the proliferating cells) had been reported from in vivo studies.

The basic studies on the cell-cycle effects of HU on the C3H mouse duodenum and the two mammary tumor lines (Slow and S102F) have been completed. In these studies, HU did demonstrate S-phase cytotoxicity and did cause a G_1/S block in all three tissues; however, evidence for partial cellular synchronization was only seen in the duodenum and the S102F tumors. The apparent lack of synchronization in the Slow tumor may be due to the long cell cycle time and low growth fraction. The degree of synchronization is low in both the duodenum and S102F tumors and desynchronization occurs quickly. In spite of this, studies involving multiple doses of HU suggested that one may use the single dose data to predict when to give subsequent doses and thereby improve the therapeutic ratio. However, to do so requires a lot of information about mechanism of drug action, tissue distribution of drug, and the recovery kinetics of normal and malignant tissues. Our own work and a review of the literature shows that the use of HU as a synchronizing agent for the enhancement of tumor therapy does not appear very promising.

3. Pulse Cytometry: A New Method for Studying the Normal and Perturbed Kinetics of Solid Tumors (Publication # 14, preprint enclosed)

The classical way for studying cellular kinetics, both in vitro and in vivo is to pulse label with ^{3}H-TdR and then prepare autoradiographs of the tissue sections or single cell suspensions and score the percent

of labeled mitotic figures. Although reasonably accurate, this method is time consuming and specifically does not apply to drug or radiation perturbed cell systems. Thus, the autoradiographic kinetic analysis that one can do following a therapeutic insult is very limited as well as tedious. Recently, however, highspeed flow systems for quantitative cytophotometry have been developed and we have collaborated with Joe Gray at the Lawrence Livermore Laboratory in studies designed to evaluate the potential of this methodology for solid tumors. In summary, there are technical problems which must be overcome (i.e., cell dispersal, non-specific staining, etc) but the methodology has exciting potential and has and will continue to be widely used for many diverse experiments.

4. Scholarly Leave (Partial support under this contract)

I had the opportunity to take a scholarly leave (July 1, 1970 - June 31, 1971) during the course of this contract and spent the time with Dr. Gordon M. Tomkins, Department of Biochemistry and Biophysics, University of California Medical Center, San Francisco. This time was intended to broaden my experience in the molecular biology of mammalian cells and in the current laboratory techniques of biochemistry and molecular biology. This year was extremely productive in this regard and is best summarized in the publications which have resulted from this work: Namely: "Further Evidence for Posttranscriptional Control of Inducible Tyrosine Aminotransferase Synthesis in Cultural Hepatoma Cells" (Pub. #6) and "Effects of Cordycepin on Cell Division, Macromolecular Synthesis, and Induction of Tyrosine Aminotransferase in Rat Hepatoma Cells in Suspension Culture" (Pub. #12).

5. Other Projects

a.) Immunological Studies:

Even though the growth kinetics of tumors from these lines are well understood in a quantitative sense, one does not know the mechanism(s) responsible for the inherently different growth rates. One mechanism which could explain some of this difference is a varying immunological response elicited by the tumors of the different lines.

This problem has been studied both by immunological suppression and attempted stimulation of the immune system. In the suppression studies, four week old mice were thymectomized and whole-body irradiated (550-650 rads, 250 Kv x-ray). Then 24 hours later, tumors from one of the lines were transplanted subcutaneously into these mice and an equal number of conventional mice. If the tumors are strongly antigenic, then one would see a higher percent of takes, a shorter latent period and a faster growth rate when transplanted into suppressed animals. A check on the degree of suppression consisted of the transplantation of a foreign tumor (BALB/c) into conventional and suppressed C3H mice, and also BALB/c skin grafts onto the C3H mice.

The second part of this study dealt with an attempt to elicit an immune response to the tumors in conventional C3H mice. Tumor fragments were transplanted subcutaneously into the right flank and allowed to grow. The latent periods and growth rates were measured and then when the tumors reached a volume of 300-400 mm^3 they were surgically removed. Ten to fourteen days later fragments from the same line were transplanted into the left flanks and growth again observed. A strong antigenic response would be noted by a lower percent of takes, a longer latent period, and lower growth rates in the second transplants.

In both cases, the results were negative. We observed no significant changes in the percent takes, latent periods, or growth rates in any of the experimental situations. Thus, the conclusion is that none of these line tumors is strongly antigenic when transplanted into C3H mice.

b.) Differentiation of C3H mouse mammary tumor cells:

Another mechanism which might partially explain the different growth rates is the extent of cellular differentiation in tumors. In 1971, I proposed to study this concept by evaluating whether tumor cells could be induced to synthesize milk proteins in organ cultures. Specifically, normal mouse mammary tissue (controls) and tumor tissue would be grown in organ culture in standard medium and in the presence or absence of insulin, corticosterone, and prolactin. These three hormones will stimulate the normal mammary gland to synthesize milk proteins, and I intended to determine to what extent, if any, this applies to tumor cells from the various lines. Organ culture was not successful. Thus, in vitro cell lines were established for both the Slow and S102F in vivo lines and some preliminary work on the effects of glucocorticoids and prolactin have been done. (See manuscript in preparation #3). This work was suspended due to lack of funds. As part of this project, the estrogen and prolactin receptor levels are being determined in spontaneous mouse mammary tumors and the Slow and S102F lines. This work is in progress and is being done as a graduate thesis by James Hinckley.

c.) Adriamycin and x-irradiation studies:

This work is an extension of the HU studies. The recovery kinetics and the clinical responses (host toxicity, tumor regression, and cures, etc.) of the duodenum and the tumor lines are being studied in depth. Although a few abstracts have been published (See #13 & 14), the work is still preliminary and will continue. Briefly, the recovery kinetics are much different from the effects of HU and the clinical response to adriamycin and x-irradiation are very different from HU and x-irradiation. This is not surprising since the mechanism of action of the two drugs is dramatically different.

EVALUATION OF PROGRESS

This contract has contributed significant information to our understanding of tumor biology and how drugs effect the cellular kinetics of normal and malignant tissues. Three areas are of special note: 1) The ^{125}I-IUdR Method, 2) The use of flow cytometry for solid tumors, and 3) The extensive recovery of kinetic data following HU administration. Much more of this type of basic information is necessary to understand the complex phenomena encountered when tissues respond to and recover from therapeutic insults.

PUBLICATIONS SUPPORTED IN PART BY THIS CONTRACT

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12. Dethlefsen, L.A.: Effects of Cordycepin on Cell Division, Macromolecular Synthesis and Induction of Tyrosine Aminotransferase in Rat Hepatoma Cells in Suspension Culture. J. Cell. Physiol. 86: 155-166, 1975.

13.* Mendelsohn, M.L. and Dethlefsen, L.A.: Effects of Selection and Passage on Volumetric Growth Rate of Mouse Mammary Tumors. Lawrence Livermore Lab. Report #UCRL-51798. April 1975. Removed

14.* Dethlefsen, L.A., Gray, J.W., George, Y.S., and Johnson, S.: Flow Cytometric Analysis of the Perturbed Cellular Kinetics of Solid Tumors: Problems and Promises. Presented at the 2nd International Symposium in Flow Through Cytophotometry, Münster, Germany, Sept. 1975 (In press). Removed

15.* Dethlefsen, L.A., Ohlsen, J.D., and Roti Roti, J.L.: Cell Synchronization in Vivo: Fact or Fancy? Presented at the 29th M.D. Anderson Annual Symposium on Fundamental Cancer Research. Houston, Texas, March 1976. (In press). Removed

Manuscript in Preparation

1. Dethlefsen, L.A. and Snively, J: ^{125}I-IUdR and Tumor Cell Loss Revisited: Cell Loss Measured from Three Established Lines of the C3H Mouse Mammary Tumor.

2. Ohlsen, J.D. and Dethlefsen, L.A.: Radiation Response of Small Intestine following Synchronization with Hydroxyurea.

3. Roti Roti, L.W. and Dethlefsen, L.A.: Interaction of Corticosterone and Prolactin on Modifying the Growth Rate of Cell Lines from Mouse Mammary Tumors.

* Copies included with this report.

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13. Dethlefsen, L.A. and Riley, R.M.: The Response of Fast-growing (S102F) and Slow-growing (Slow) C3H Mouse Mammary Tumors to Adriamycin (AM) and X-irradiation (x-r). Rad. Res. 62: 558, 1975.

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TRAINING SUPPORT

This Contract has supported, in part, the training of two veterinary students in the Summer Research Program, one third-year undergraduate from Lincoln University, and two minority high school students from Philadelphia. These programs were designed to introduce the students to careers which are available in biomedical research and to give them actual experience in a research environment.

Dr. L. Roti Roti, Associate Instructor; Steve Johnson, and Jim Hinckley, both graduate students in Anatomy; and Dr. Joel Ohlsen, a radiotherapy resident have contributed to this project at the University of Utah. None of these people received financial support from this contract.