

OFFICE MEMORANDUM

712660

TO : M. M. Kligerman - ADRT 8/28/72

DATE: August 25, 1972

FROM : C. R. Richmond

SUBJECT: OPERATIONAL GRANT FOR THE CANCER RESEARCH AND TREATMENT CENTER

SYMBOL : H-4

The attached information has been prepared by Group H-4 staff in response to your request of 14 August related to the memorandum subject. The areas of potential collaboration are general rather than specific because of the difficulty in anticipating just how efficiently such collaborative work could be implemented in terms of staff and current program responsibilities. However, the attached information should give you much of the material you requested.

We have not included information under the budget category, as we consider the proposed areas of research to be, in general, parts of ongoing programs that might provide very general points of common interest for collaborative work with the Cancer Research and Treatment Center.

We look forward to the possibility of collaborative research projects and to the future success of the Cancer Research and Treatment Center.

C. R. Richmond

CRR:ES
Enc. 5 project areas

CC:G. L. Voelz - H-DO
Enc. (a/s)

*Cy To N D Blum, 8/30/72
From C R Richmond*

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BIOPHYSICSAim

To apply the unique instrumentation capability developed at the Los Alamos Scientific Laboratory to problems of detection, identification, and isolation in tumor model systems and human neoplasms.

Research Plan

Mammalian cells possess numerous physical properties which can be exploited to permit rapid classification on the basis of parameters such as size, genome content, nuclear-to-cytoplasmic ratio, and surface receptor specificity. Instrumentation developed and available at the LASL is capable of the following:

- (1) Conductimetric estimation of cell volume using viable material and physical separation of selected cell sizes from a volume distribution.
- (2) Measurement of the genome content of individual cells stained by fluorescent-Feulgen reactions (acriflavine, auramine-O) or intercalation (ethidium bromide) at high speed in a laminar flow fluorescence detection system, flow microfluorometry (FMF). The system is capable of making 10^4 to 10^5 individual measurements in minutes and will perform absolute genome measurements and convenient life-cycle analysis with proper data processing.
- (3) Measurement of surface staining properties dependent on cell-surface receptors for fluorescent antigens and plant lectins by FMF techniques at high speed.
- (4) Estimation of nuclear-to-cytoplasmic ratios and variations in cytoplasmic inclusions by light scattering techniques.
- (5) Multiparameter physical separation of cells possessing aberrant

genome content, nuclear-to-cytoplasmic ratio, or absolute volume capable, for example, of automated screening of vaginal and bronchial exfoliative cytology specimens.

Significance

Within the framework of specific research plans, this instrumentation provides unprecedented statistical precision and convenience and permits the investigator to contemplate experiments heretofore not feasible technically. Use of this instrumentation for routine screening and diagnosis in the context of application in a Cancer Research and Treatment Center is unique.

BIOCHEMISTRYAim

To examine details of the regulation of translation in normal and malignant cells.

Research Plan

Over the past several years, cell-analysis systems developed at the LASL permit extensive characterization of the nucleic acid and basic protein species in synchronized populations. Details of biosynthesis and modification are now sufficiently well known to permit comparative studies of normal and transformed cells in culture. Extension of these studies is potentially useful for evaluating effects of chemotherapeutic agents and radiation on biosynthesis and cell-cycle traverse. Specific experiments are designed to test the differential effect of radiation on initiation and continuation of DNA replication, translation rate, and histone biosynthesis and effects of chemotherapeutic agents primarily on cell-cycle progression as a model for predicting the efficacy of specific agents in the treatment of human neoplasms.

Significance

These studies represent extension of a productive ongoing program in tumor cell biology. For example, we have shown that, once DNA synthesis is initiated, radiation does not interfere with its completion and that H-thymidine incorporation is an inaccurate measure of DNA synthetic rate following irradiation because of pool effects. Contemporary chemotherapy places great emphasis on cell-cycle effects using the argument that cells have periods of enhanced sensitivity

which are cell-cycle-dependent. These studies are specifically designed to determine the effects of new agents on cycle progression, and clinical applications are being designed on the basis of this information.

RADIATION BIOLOGYAim

To explore problems in radiobiology and radiotherapy related to the relative biological effectiveness (RBE) and carcinogenesis resulting from high LET radiations.

Research Plan

The idea that high LET radiations occupy an advantageous position in treatment of human neoplasms currently is being investigated in a series of experiments preceding a clinical trial of the therapeutic efficacy of negative π mesons. However, the interactions of negative π mesons are sufficiently complicated to defy precise prediction of their biological effects in complex systems (neoplasms in man). Therefore, we propose to investigate model systems [i.e., cells in culture exposed to high LET radiations (^{238}Pu , ^{239}Pu , and alpha particles) and low LET radiations (250 KVP x-rays)] to characterize (1) effects on cell-cycle times; (2) survival; (3) initiation and continuation of genome replication; and (4) effects on immediate and protracted genome stability in survivors by examination of trypsin-induced Giemsa banding in metaphase chromosomes. These studies will employ currently used methodology in a laboratory which is qualified to fabricate and handle plutonium alpha sources.

An ongoing program is directed toward description of cytological events occurring in the lungs of hamsters containing multiple plutonium point sources of varying radiation intensity. The development of neoplasms under these experimental conditions offers the opportunity to investigate ancillary questions concerning malignant transformation in organized systems. The most generally accepted contention is that primary neoplasia is the result of transformation

of a single cell in a population which consists of cells in cycle, in G_0 , and in which a series of metaplastic alterations suggests that a significant proportion is dead or dying and in which immuno-competence is either temporarily or permanently altered. Under conditions where the cell-cycle parameters and viability of the geometric population at risk are known, immuno-suppressed animals will be exposed to a known lung burden of plutonium particles, and the frequency and histologic type of induced neoplasms will be evaluated.

Significance

Contemporary opinion concerning the therapeutic efficacy of high LET radiation is guardedly optimistic, but the opinion is based largely on empirical observations which provide little information on basic cellular responses crucial for optimizing treatment schedules, assessing late effects, genetic effects, or mechanisms of action. These experiments indicate the direction in which an ongoing and productive program will be expanded. Collaboration with the Cancer Research and Treatment Center might be mutually beneficial.

IMMUNOLOGYAim

To investigate cell-surface properties of cultured material and circulating lymphocytes.

Research Plan

Cell-surface properties of cultured material and circulating lymphocytes in vivo indicate that they have many macromolecular species in common, and comparative investigations of binding of specific fluorescent antigens and fluorescent conjugated plant lectins (concanavalin A, wheat germ, etc.) will be approached as an appropriate quantitative model for characterization of cell-surface receptor sites. The experiments involve distribution and surface staining of cycling material where both the cycle phase distribution and surface staining intensity are quantitatively monitored by flow microfluorometry techniques.

According to Dr. L. Hempelmann of the University of Rochester, an excessive frequency of auto-immune diseases is appearing late in the lifetime of patients irradiated as children in the treatment of "thymic hypertrophy." These patients, as well as patients treated for breast cancer, show a high preponderance of B lymphocytes as judged by their agglutination response to sheep red cells and PHA stimulation in culture. Dr. G. Bell (LASL) has proposed a model for the proliferation and kinetic behavior which can be approached experimentally by distinguishing between T and B lymphocytes employing fluorescent flow techniques. The T and B lymphocyte distributions will be obtained following both experimental and

therapeutic radiation, in an attempt to extend understanding of post-radiation immuno-competence.

Significance

These studies offer the possibility of placing investigation of cell-surface properties and immuno-competence on a truly quantitative basis. Binding kinetics measurements permit a detailed understanding of the interactions of cell-surface receptors with a variety of agents (i.e., topography, ontogeny, immuno-paralysis, etc.). The studies are particularly attractive because they combine strong theoretical capability with unique experimental methodology.

CYTOGENETICS

Aim

The application of newly developed techniques for morphological and biochemical monitoring of the genome in mammalian cells to facilitate description of normal and malignant populations.

Research Plan

The Biomedical Research Group has shown that high heteroploid cells in culture exhibit a greater degree of constancy of DNA content than would be expected on the basis of variability in chromosome number. This observation, which implies a high degree of genetic homogeneity in tumor cell lines, has been confirmed by a modified Giemsa banding technique which clearly shows that, despite extensive rearrangement, the euploid genome has been almost quantitatively retained during the transition from euploidy to aneuploidy. Furthermore, these observations rule out nondisjunction as a mechanism for heteroploidization and strongly suggest acquisition of excess genome as entire haploid sets; the first step is almost certainly a cytokinetic defect. We propose to extend these studies to the following basic areas of inquiry: (1) to determine the extent of karyotypic rearrangement in established human tumor lines and a large number of primary human tumors, and (2) to further characterize the regulatory constraints on transformed cells. Preservation of the template-active genome appears to be essential both in amount and organization.

Significance

The modified Giemsa banding technique is highly reproducible, rapid,

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simple, and adaptable to automation. Therefore, it is applicable not only to basic problems in tumor biology but also to routine examination of human material which can be cultured. The utility of the method in assessing genetic homogeneity has been demonstrated, and it offers much greater sensitivity and resolution for detection of rearrangements in the genome of tumor cells than do conventional techniques. New Mexico has one of only two tumor registries in the nation which effectively monitors all presenting cancer cases in the state's population. Thus, it provides the opportunity to screen large numbers of accurately diagnosed patients to determine whether diagnostically useful information can be obtained and at the same time provide material for testing the generality of notions concerning the relative homogeneity of specific tumor types within the population.