BIONETICS RESEARCH LABORATORIES, INC. (NIH 71-2025)

Title: Investigations of Viral Carcinogenesis in Primates

Contractor's Project Director: Dr. Harvey Rabin

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber
Dr. Gary Pearson

Objectives: (1) Evaluation of long-term oncogenic effects of human and animal viral inocula in primates of various species, especially newborn macaques; (2) maintenance of monkey breedir colonies and laboratories necessary for inoculation, care and monitoring of monkeys; and (3) biochemical studies of transfer RNA under conditions of neoplastic transformation and studies on the significance of RNA-dependent DNA polymerase in human leukemic tissues.

Major Findings: This contractor continues to produce over 300 excellent newborn monkeys per year. This is made possible by diligent attention to reproductive physiological states of female and male breeders. Semen evaluation, artifical insemination, vaginal cytology and ovulatory drugs are used or tried as needed.

Inoculated and control infants are hand-fed and kept in modified germ-free isolators. They are removed from isolators at about 8 weeks of age and placed in filtered air cages for months or years of observation. The holding area now contains approximately 1200 animals up to 5 years old. Approximately 300 are culled every year at a rate of about 25 per month. This is necessary to make room for young animals inoculated with new or improved virus preparations.

New importance is being given to the New World species of monkeys, including squirrel, marmoset, and spider monkeys. Animals currently on study are being actively culled to reflec this change.

Special emphasis has been placed on virological studies characterizing the Mason-Pfizer monkey virus (M-PMV). Seven sublines established from chronically M-PMV-infected rhesus foreskin cultures were shown to be releasing moderately high titers of infectious M-PMV, and in addition seemed to have undergone in vitro transformation. Inoculation of cells of these sublines into newborn rhesus monkeys produced palpable masses at the sites of inoculation. Biopsies performed on these masses and on the regional lymph nodes of the same animals revealed the presence of proliferating virus characte istic of M-PMV by both electron microscopic and cell culture

analysis. Proliferating M-PMV was found in the lymph nodes of monkeys inoculated with cell-free M-PMV preparations.

Chromatographic examination of transfer RNA's (tRNA's) from control and virus-transformed rat and mouse embryo cells demonstrated differences in phenyl-alanyl-tRNA's and aspartyl-tRNA's. No differences were noted in the elution profiles of seryl-, tyrosyl-, leucyl-, asparaginyl-, or glutaminyl-tRNA.

The effects of 11 rifamycin derivatives on viral reverse transcriptase and on DNA polymerases from human normal and leukemic blood lymphocytes were evaluated. Compound 143-483, 3-formyl rifamycin SV: octyl oxime showed the greatest potency and inhibited all DNA polymerases from both viral and cellular origins.

The contractor also engaged in collaborative studies involving the oncornavirus, RD-114, from a human sarcoma, isolated by Drs. McAllister, Gardiner, and Huebner. The virus is being produced and supplied by Dr. Gilden of Flow Laboratories. Another virus, a human papovavirus associated with progressive multifocal leukoencephalopathy, is being supplied by Dr. Duard Walker for inoculation into newborn monkeys.

Significance to Biomedical Research and to the Program of the Institute: Inasmuch as tests for the biological activity of candidate human viruses will not be tested in the human species, it is imperative that another system be developed for these determinations and. subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes. Further study of altered transfer RNA and polymerase enzymes would determine their significance in neoplastic change and provide a basis for selection of therapeutic agents.

Proposed Course: The previously mentioned studies will be continued and expanded. Particular attention will be given to research on animals inoculated with candidate human cancer viruses, and investigations will be carried forward into the nature of neoplastic changes and their possible control at the cellular level. Collaborative efforts with other researchers within the SVCP will continue.

Date Contract Initiated: February 12, 1962

Current Annual Level: $2,153,850

Merck and Company, Inc. (NIH-71-2059)

Title: Study of Viruses in Human and Animal Neoplasia.

Contractor's Project Director: Dr. Maurice R. Hilleman

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Jack Gruber

Objectives: To perform investigations designed to develop vaccines or other agents effective for the prophylaxis and therapy of human neoplasia of suspected viral etiology.

Major Findings: This is a new contract.

Significance to Biomedical Research and the Program of the Institute: Current data support the concept that a virus or viruses are the essential element in most animal tumors studied and that viruses are probably the necessary etiological component in human neoplasia, though expression may be greatly influenced and modified by host and environmental factors. If viruses are the essential element in human cancer, then prophylaxis by vaccines to prevent or minimize infection should provide a rational approach to cancer prevention. This could be accomplished by utilization of live or killed virus vaccines or possibly by vaccines of purified virion subunits.

Vaccines would obviously provide their greatest benefit in preventing infection with oncogenic viruses transmitted horizontally after birth. However, even the possible vertical transmission of hypothetical neoplastic agents does not rule out a potential benefit from vaccines. Nononcogenic viruses may function as essential cofactors in expression of neoplasia, and immunity against such secondary agents might prevent expression of the neoplastic state. Additionally, antibody or cellular immunity may be enhanced by vaccination with homologous virus in virus-dependent cancer. Obviously this research investigation is of fundamental importance to the goals of SVCP and can make unique contributions to the total program.

Proposed Course: The investigators will devote initial efforts to developing methods for propagation, purification, concentration and specific quantitation of candidate viruses suspected or shown to cause cancer in man. At the present time, investigations will be focused upon herpes-type (DNA) viruses and "B" and "C" type (RNA) particles. Parallel studies to evolve live attenuated and killed virus vaccines in appropriate animal model systems will be conducted. Particular attention will be given to developing and applying optimal methods for viral attenuation, viral inactivation, viral quantitation, vaccine safety assessment, and vaccine potency assay.

Date Contract Initiated: March 1, 1971

MERCK AND COMPANY, INC. (NIH-71-2059)

Title: Oncogenic Virus Research and Vaccine Development

Contractor's Project Director: Dr. Maurice Hilleman

Project Officers (NCI): Dr. Robert A. Manaker
Mr. J. Thomas Lewin

Objectives: To conduct investigations designed to develop vaccines or other agents effective for the prophylaxis and therapy for human neoplasia of suspected viral etiology.

Major Findings: Multiple construction and renovation projects have been involved in the expansion and reorientation for this program. Remodeling of a laboratory, physically separated from the animal tumor virus area, was recently completed and is in use for Herpes simplex type 2 vaccine work. Two rooms (440 sq. ft.) in Bldg. #43 were remodeled and equipped and are in use for the germ-free derivation of kittens for the SPF cat colony breeding nucleus. Plans were completed for the renovation of half of Bldg. #65 (5,940 sq. ft.) for housing an SPF cat colony and for housing experimental cats. The construction and equipping of the new biohazard containment building #26B (12,096 sq. ft.) for laboratory work is progressing on schedule. The projected completion date is September, 1972.

Tumor-specific cellular vaccine development: The preparation and assay of tumor cell vaccines for protective efficacy in the hamster model system was continued at a lower priority level. Testing of adenovirus 31 tumor cell fractions prepared by mechanical disruption of the cells and fractionation by differential centrifugation was completed. None of the vaccines (crude cell homogenate, nuclear fraction-$\omega^2 t = 10^7$ pellet, membrane fraction-$\omega^2 t = 5 \times 10^9$ pellet, particulate fraction-$\omega^2 t = 10^{11}$ pellet, cell sap-$\omega^2 t = 10^{11}$ supernate) protected hamsters against development of tumors when they were challenged by inoculation of viable homologous tumor cells. Work on the preparation of two other types of tumor cell antigens was continued. Cell membranes were prepared from a adenovirus 12 tumor cells by hypotonic extraction and were solubilized by sonication. The solubilized material was fractionated on Sephadex G200

columns and the desired fraction concentrated by the Diaflo membrane technique. The first batch of test and control antigens is on test for protective efficacy in hamsters. Preparation of additional batches of antigen for assay is in progress. Technology is still being developed for the preparation of adenovirus 7 tumor cell membranes by flow sonication and flow zonal centrifugation.

Investigation of the host immunologic response to nonprotective tumor cell vaccines is being conducted in hamster-tumor model systems. The first series of experiments was designed to test the effect of inoculation of known nonprotective vaccines before, simultaneously with, or after immunization with a known effective vaccine (5×10^6 γ-irradiated tumor cells). Most of the experiments in this series are on test. Final results with one of the nonprotective vaccines, SV_{40} tumor cell ghosts prepared by hypertonic extraction, showed that this vaccine did not interfere with the ability of the host to reject viable homologous tumor cells after vaccination with 5×10^6 γ-irradiated SV_{40} tumor cells.

Attempts to render nonprotective SV_{40} tumor cell vaccines effective by the administration of poly I:C before, simultaneously with, or after vaccine, single or multiple doses, or by different routes were not successful in the hamster model system.

Studies on the role of fetal antigens in tumor immunology are being conducted in the SV_{40}-hamster model system. In the first series of experiment, γ-irradiated, 9-12 day gestation fetal cells of multiparous origin did not protect adult male or female hamsters against tumor development when challenged with 5000 homologous tumor cells. Experiments are in progress wherein the vaccines were prepared from primaparous 10-day gestation embryos and are being tested in the SV_{40} virus-newborn hamste model system and in the adult hamster-tumor cell challenge system with a 2500 cell challenge dose.

Virus vaccine development: This project is still in the initial stages. The work in progress is concerned primarily with basic needs such as virus propagation, virus concentration and purification, preparation of specific antisera, and establishment of routine assay procedures.

The KT (Kawakami-Theilen) strain of feline leukemia virus (FLV) was routinely propagated in roller bottle (1 liter/bottle) suspension cultures of the virus-shedding FL74c cell line. Ten liter lots of culture fluid were concentrated (1000x) and purified by flow zonal centrifugation and isopynic centrifugation on sucrose gradients. Modifications in technology are still being made to increase the purity of the concentrated virus. Virus yields of 10^{13} virus particles/ml were readily achieved.

In order to provide an adequate supply of healthy cats for future experimental work, establishment of a specific pathogen-free cat colony was proposed. The first step, the germ-free derivation of the breeding

has been in progress for two months. All eight isolators are occupied by kittens (16 females, 7 males) ranging from 1 to 8 weeks in age.

Significance to Biomedical Research and the Program of the Institute:

If viruses are an essential element in the genesis of some human cancers, prophylaxis by vaccines to prevent or minimize infection should provide a rational approach to cancer prevention. This could be accomplished by living or killed virus vaccines or possibly by vaccines of purified virion sub-units. Although greatest benefit could be derived by prevention of infections transmitted horizontally after birth, a potential benefit from vaccines may be derived where viruses are transmitted vertically but do not express their full antigenic complement. Non-oncogenic viruses may function as essential co-factors in expression of neoplasia, and immunity against such secondary agents might prevent expression of the neoplastic state. In addition, vaccination with homologous virus in a virus-dependent cancer may enhance specific humoral antibody or cellular immunity. This research project is of fundamental importance to total program.

Proposed Course: Efforts to prepare tumor-specific cellular antigens for immunoprophylaxis of cancer and to study the immunologic response to such antigens will continue. Tests with poly I:C for adjuvant effect on ineffective cellular vaccines will be completed. Work towards development of a feline leukemia-sarcoma virus vaccine and a herpesvirus type 2 vaccine will be continued as rapidly as possible. If no problems arise, the germfree derivation of kittens for the SPF cat colony should be completed in several months.

Date Contract Initiated: March 1, 1971

Current Annual Level: $1,016,000