Fig. 8.6. Baylor College of Medicine Cancer Contract

CONTRACT SUMMARIES

SPECIAL VIRUS CANCER PROGRAM
ETIOLOGY AREA, NCI
Fiscal Year 1971

DEVELOPMENTAL RESEARCH PROGRAM SEGMENT

Dr. Robert A. Manaker, Chief, VBB, Etiology Area, Chairman
Dr. Roy F. Kinard, VBB, Etiology Area, Vice Chairman
Dr. Jack Gruber, VBB, Etiology Area, Executive Secretary[1]

AICHI CANCER CENTER (NIH-69-96)

Title: Virus Rescue Studies in Human Leukemia/Lymphoma Cell Lines

Contractor's Project Officer: Dr. Yohei Ito

Project Officers (NCI): Dr. Jack Gruber
Dr. Virginia C. Dunkel

Objectives: (1) To establish cell lines in vitro from human neoplasms and examine these for virus or antigens by electron microscopy, immunology and transformation experiments. (2) To supply human embryonic cell cultures and lymphoma-type tumor tissues available in the Far East.

Major Findings: Efforts to establish continuously growing cell lines from human neoplastic tissues were resumed as one of the main lines of study in the second year of the contract. In addition to the cultures from neoplasia of the hematopoetic system, cell cultures from solid tumors such as nasopharyngeal carcinoma (NCP) were also attempted. This was done because of the well established fact of high herpes-type virus (HTV) antibody titer in the sera of patients with the disease. Among 13 NPC specimens cultured, 8 gave rise to monolayer culture, of which half showed morphological alteration. From these cultures, one free-floating cell line was established. The presence of HTV antigen in these cells was demonstrated by direct immunofluorescence test.

To obtain an established cell line which grew in a floating state with less or hopefully no HTV antigen, cultures from hyperplastic tonsils of children were carried out. Of 136 specimens, 12 cell lines were established as free-floating cells. The ratio of cells containing HTV antigen was relatively small but HTV antigen was detected in all the cell lines.

Some 50 strains of cells were maintained in the laboratory and they served as a procurement center for the supply of the cells for research workers in the area. The procurement of human embryonic cultures was also continued into the second year. About 40 human embryos in total were processed for such culture. Human sera of high HTV antibody titer were also supplied to colleagues of the SVCP.

[1] Replaced Dr. Roy Kinard as Vice Chairman on March 2, 1971.

Seroepidemiological studies using indirect immunofluorescence test have been continued to accumulate more data on the HTV antibody titer of individuals with various neoplastic diseases and normal subjects. However, a hope to reveal a new disease with high HTV antibody titer turned out to be fruitless so far.

Contacts have been strengthened with the institutes of the Asiatic area to provide access to the human tumor material and serum specimens which might be useful to the SVCP.

Significance to Biomedical Research and the Program of the Institute: This project will supply supporting data from Far Eastern sources to supplement information obtained in the U.S. on the association of viruses with specific neoplastic diseases.

Proposed Course: In general, studies initiated previously will be continued. A new aspect of the work scope is the introduction of biochemical techniques to search for the presence of RNA-dependent DNA polymerase among the approximately 90 cell lines established from human neoplastic tissue during the past two years. Furthermore, fresh human cell materials from leukemic and lymphoma patients at the Aichi Cancer Center Hospital will be tested for polymerase activity. Such studies would provide new data on neoplastic cells from patients of oriental origin. Additionally, plans are to study the in vitro effect of various chemical carcinogens on established lymphoblastoid cell lines, and to initiate new investigations on other human neoplasms where virus activity is suspected.

Date Contract Initiated: May 2, 1969.

BAYLOR COLLEGE OF MEDICINE (PH43-68-678)

Title: Studies on Viruses as Related to Cancer with Emphasis on Leukemia

Contractor's Project Director: Dr. Joseph Melnick

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

Objectives: (1) To isolate, propagate and identify viral agents to provide evidence of association with human neoplasia and (2) to continue to hold and observe primates inoculated with suspected cancer viruses or cancer tissues.

Major Findings:

A. Viral etiology of leukemia and mononucleosis.

Propagation of selected lymphoblastoid (Ly) cell lines from patients with leukemia or mononucleosis and normal individuals is continuing, for use in immunological and biophysical studies.

Comparative chromosomal analysis of 16 lymphoblastoid cell lines cultivated up to 61 months revealed no specific association between the presence of EB virus and any of the chromosomal anomalies observed. Regardless of the source of cells or the presence of EB virus, cells with marker chromosomes or trisomies appear to have a selective advantage of growth in vitro, as documented by the increase in frequency of clones with these anomalies with progressive passage.

Studies were continued to search for antibody to possible tumor antigens in acute leukemia of childhood. Peripheral blood leukocytes are being reacted with serial autologous serum samples by indirect membrane immunofluorescence (IMIF). In autologous tests, cells from 20 of 25 children in remission have yielded negative results and 5 questionable. Cells obtained from 6 children during relapse were also tested for autoantibody. The IMIF assay has been uniformly negative for all sera and autologous relapse phase cells tested so far.

A 78 A1 cell line of rat embryo fibroblasts that had been transformed by the murine sarcoma-leukemia virus (MSV-MLV) complex is being used as a model for characterization of RNA species obtained from various human Ly cell lines. The 68S RNA of MSV-MLV was found to dissociate after heating or dimethylsulfoxide treatment into 37S, 18S and 4S subunits, differing in base composition and buoyant densities in cesium sulfate. A double-stranded DNA with a sedimentation coefficient of 7S density was isolated from highly purified MSV-MLV. This DNA was complementary to the 18S subunit, but not to the 37S or 4S subunits of the viral RNA. Work is being initiated to follow infectious virus synthesis and macromolecular synthesis during de novo infection and transformation of cells by MSV-MLV.

B. Comparative studies on herpesviruses.

Studies continue on the distribution of complement-fixing (CF) antibody to EB virus-induced S antigen in groups with various disease entities and in normal individuals. The results are compared with those obtained in the immunofluorescence (IF) test with fixed EB3 cell preparations. Sera from 21 patients with sarcoidosis, kindly supplied by Dr. Phillip Glade, revealed both IF and S antibody to EB virus, a result similar to that reported earlier for patients with post-nasal carcinoma. A longitudinal study of the development of IF and S antibody starting with newborn children is being conducted. The results to date indicate that: (1) IF antibody to EB virus appears within several months after maternal antibody has disappeared, indicating an early infection with this agent. (2) There is a lag of several months between the appearance of IF and S antibodies. (3) Infection with the other three herpesviruses (herpes simplex, cytomegalovirus and zoster virus) occurs much later than infection with EB virus.

Work continues to purify and characterize the soluble CF antigens extracted from two EB virus-positve (EB3 and P3J) and from one EB virus-negative (NC37) Ly cell lines. Ly cell line RPMI 8226, free of both EB virus and soluble CF antigen, is used as negative control. Studies on the non-serum-requiring complement-fixation (NSR-FCF) by EB virus-positive Ly cells

indicate that the reaction may be measuring an EB virus directed antigen-antibody. Attempts are being made to identify antibody to EB virus in extracts of Ly cells.

The biochemical and biophysical properties of the DNA of HSV type 1 and type 2, infectious bovine rhinotracheitis (IBR) and Pseudorabies (Pr) viruses were compared. DNA-DNA hybridization was employed to investigate genetic relatedness between these four members of the herpesvirus group. Saturation and competition hybridizations demonstrated greater than 90% homology between three strains of HSV-1 and less than 5% homology between HSV-1, Pr virus and IBR virus. Saturation hybridization indicated at least 90% homology between two strains of HSV-2, but no homology between HSV-2 Pr virus and IBR virus. Preliminary experiments indicate that there is less than 50% homology between the DNAs of type 1 and type 2 HSV.

Further attempts to obtain additional BUDR-induced ts mutants of HSV have resulted in the isolation of 50 potential mutants. The 22 original ts mutants of HSV have been tentatively assigned to 8 complementation groups. Groups so far contain from 1-4 mutants. Before detailed characterization of representative members of complementation groups is undertaken, repeat tests are planned using both the 22 original mutants and the newly isolated mutants mentioned above.

The protein and glycoprotein synthesis by a HSV temperature-sensitive mutant (ts 343) and the wild-type virus has continued to be compared by polyacrylamide gel electrophoresis. Evidence was obtained that mutant ts 343 does not synthesize glycoprotein C5, a major envelope protein, at the nonpermissive temperature (40°). Studies are in progress on the glycoprotein synthesis of additional HSV ts mutants. Preliminary findings indicate that the glycoprotein profiles of mutants belonging to separate complementation groups may be significantly different.

C. Role of herpes virus type 2 in cervical carcinoma.

Seroepidemiologic studies of women with invasive cervical cancer and controls from Uganda and Israel failed to reveal the same strength of association between antibodies to herpesvirus type 2 and malignancy, as previously observed in Houston, Atlanta, Baltimore and Brussels. Studies in different populations are continuing. Recently, 118 cervical cancer patients and 83 controls from Muslim and Christian women in Yugoslavia were studied. No difference in type 2 herpes antibodies was found between Muslim cases and controls, but the occurrence of antibodies among Christian women was twice that of control women. In New Zealand, the occurrence of antibodies was only slightly higher in the patients than in the control women. In the USA most of the studies that yielded a high incidence of antibodies were carried out among women of the lower socioeconomic group. Thus, in populations of different composition and different life styles, differences are found in the association between type 2 herpesvirus and cervical carcinoma.

Type 1 strains are susceptible to cytoside arabinoside and IUDR (iododeoxy-uridine), inhibitors of replication of DNA-containing viruses. However, type 2 strains are resistant to these DNA antagonists. This is apparently due to the low levels of thymidine kinase which are characteristic of type 2 strains in contrast to the high levels of this DNA-synthesizing enzyme found with type 1 strains.

D. Immunofluorescent cell surface antigens in human tumors.

Cell lines have been established from human melanoma and sarcoma tissue. Attempts are being made to separate tumor cells from the normal cells by (1) density gradient centrifugation, (2) by cloning out tumor cells, and (3) by injecting the mixture into immunosuppressed mice. With one of the two melanoma cell lines tested, the patient's serum reacted in the membrane fluorescence test. Sera from other melanoma patients reacted positively for antigen in the cytoplasm of the tumor cells. Lymphosarcoma cells reacted in the membrane fluorescence test with autologous sera, and cross reactions were seen with sera from other lymphosarcoma patients.

Significance to Biomedical Research and to the Program of the Institute: This contract provides a progressive comprehensive research program to determine the significance of viruses in human neoplasia. Techniques used are tissue culture, immunology, electron microscopy, primate inoculation, cytogenetics and nucleic acid homology.

Proposed Course: Investigations will continue to detect the presence of nucleic acid characteristic of the RNA tumor viruses in human tumor cells. Immunological studies with antigens associated with EB virus will be continued. Further serologic data will be acquired to aid in determining the relationship between the venereal herpes hominis type 2 virus and cervical carcinoma.

Date Contract Initiated: June 27, 1963

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

Title: Investigations of Viral Carcinogenesis in Primates

Contractor's Project Directors: Dr. John Landon
Dr. David Valerio
Dr. Robert Ting

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber
Dr. Robert Gallo

Fig. 8.4. Medical Experiments Done on Huntsville Prisoners

TABLE 1

Summary of Research Programs Conducted By
Baylor University School of Medicine

Study	Number of Inmates	Date
Hong Kong flu program	500	12-24-68
Flu - influenza vaccine	37	1 -69
Rhinovirus 353 vaccine	130	3-11-69
Adenovirus vaccine	43	7-22-69
Adenovirus vaccine	13	7-24-69
A/Z Hong Kong flu	9	7-26-69
Equine flu study	10	7-26-69
Adenovirus 5 challenge	58	9-27-69
Influenza	111	11-08-69
Blood draw	46	1-27-70
Parainfluenza study	55	5-29-70
Mycoplasma pneumonia vaccine study	46	9-10-70
Rhinovirus type 15 plague pool	55	9-10-70
Parainfluenza	37	3-17-71
Mycoplasma pneumonia hall study	116	5-19-71
Adenovirus vaccine study	15	5-19-71
X-32 vaccine hall study	4	6-13-71

TABLE 1 (Continued)

Study	Number of Inmates	Date
Virus	12	9-24-73/10-23-73
Virus	15	8-13-73/9-10-73
Blood donor	5	11-14-73
Blood donor	482	10-31-73
Blood donor	11	1-08-74
Influenza/virus	61	12-06-73/1-06-74
Adenovirus	77	12-16-73/1-13-74
X-38 vaccine study	16	11-09-73/12-07-73
Blood donor	11	2-01-74
Virus study	5	2-06-74
Virus study	419	2-07-74
Blood donor	55	2-14-74
Adenovirus blood donor	16	2-12-74
Parainfluenza study	29	3-11-74/4-07-74
M. pneumonia	20	3-11-74/4-14-74
GCRC	14	2-25-74/3-25-74
Blood donor	29	4-27-74
Mycoplasma pneumoniae	46	4-28-74/6-02-74
Blood donor	7	5-29-74
GCRC	10	5-06-74/6-03-74
Adenovirus vaccine	12	5-10-74

Fig. 8.5. University of Texas Cholera Studies On Huntsville Prison Inmates.

TABLE 2

Summary of Research Programs Conducted By
University of Texas Medical Branch at Galveston

Study	Number of Inmates	Date
Cholera H-3	180	4-67/4-68
Cholera H-4	104	5-68/1-69
Cholera H-5	171	9-68/1-69
Cholera H-6	113	7-70/9-71
Cholera H-7	74	10-70/2-72
Cholera H-8	98	1-73/2-74
Cholera H-9	---*	10-73
TOTAL 6	740	4-67/2-74

*Study in progress.

Fig. 8.7. Huntsville Civilians* Killed and Cited Cause of Death

1. Susan Baldy	ALS
2. James Brian, Esq.	ALS
3. Georgia Stockman	ALS
4. David Ward	ALS
5. James Selford	ALS
6. Jean Harold	ALS
7. Mary Ertz	ALS
8. Barbara Rango	ALS
9. Luther Antler	ALS
10. Ken Stalworth	ALS
11. Eula Andrew	ALS
12. Evelyn Baken	ALS
13. Saul Barr	ALS
14. Robert Carry	ALS
15. Velma Enver	ALS
16. Edwin Garrison	ALS
17. Katheryn Kravitz	ALS
18. Gwen Robertson, RN	Leukemia
19. Carolyn Wishert	Cancer
20. Huston Carrie	Leukemia
21. Bernice Getty	ALS
22. Gracey Dunning	ALS
23. Polly Langley	ALS
24. Wallace MacMillan	ALS
25. Edward Naples	Brain Tumor
26. Thomas Green	ALS
27. Louis Pelling	Unknown
28. Helen Albert	Unknown
29. Joseph Cally, DDS	Heart Burst
30. Eugene Akerly, MD	Unknown
31. J.B. Sally	Brain Tumor
32. Sam Baldy	Unknown
33. Christine Angler	Unknown

Total Dead	42
Cause of Death Cited:	
ALS	25
Cancer	7
Mycoplasma fermentans incognitis	1
Cause Unknown	8
Heart	1

* Sample listing of forty-two (42) dead. All aliases cited.
For more information contact attorney Northrop @ 405-946-8100; Fax 405-949-2172

Fig. 8.8. Huntsville's Sick Plaintiffs*, Diagnosis, and Summary

1. Sally Meely	Mycoplasma
2. Clarence Meely	Mycoplasma
3. Julie Jackson	Mycoplasma
4. Nancy Messler	CFIDS
5. Marie Frankel	CFIDS
6. Larry Ryan	Lupus
7. Jerry Ryan	Lupus
8. Jackie Clarry, RN	MS
9. Robert Brandish	Cancer
10. Cherry Hartley	Fibromyalgia
11. Carol Derry	Crones
12. David Derry	MS
13. Mary Lou Perry	Mycoplasma
14. Ken Perry	Mycoplasma
15. Betty Curtan	Fibromyalgia
16. Carol Chamas	Fibromyalgia
17. Janice Selley	Cancer
18. Charlie Spinney	Cancer
19. Eugene Orlanda	Legionella
20. Virginia Arder	Epstein Barr
21. Dianne Nelly	Lupus
22. Dorothy Merrie	Parkinson's
23. Amy Ornstein	Fibromyalgia
24. Mitchel Bates	MS
25. Sheryl Horner	CFIDS
26. Virginia Latley	CFIDS
27. Jeanie Orders	CFIDS
28. Jason Brownstein	Polymyositis
29. Jackie Brownstein (Grandaughter)	Meningitis
30. Earl Jenson (child)	Joint Disease

Total Ill:	231
Diagnosis Cited:	
CFIDS	71
Mycoplasma	13
Fibromyalgia	10
Lupus	8
ALS	4
Cancer	9
MS	13
Various	23
Undiagnosed	80

* Sample listing of 231 ailing. All aliases cited.