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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

February 4, 1982

NATIONAL INSTITUTE OF
ENVIRONMENTAL HEALTH SCIENCES
P.O. BOX 12233
RESEARCH TRIANGLE PARK, N.C. 27709
(919) 541-7724

Re: 1 R01 ES02892-01 SSS

Dr. Guy David Griffin
Health and Safety Research Division
Oak Ridge National Laboratory
P.O. Box X, Building 4500S
Oak Ridge, Tennseess 37830

Dear Dr. Griffin:

The National Advisory Environmental Health Sciences Council, at its January 25 meeting, did not recommend approval of your research grant application referenced above. You may be assured that this action will not affect the full consideration of other applications you may want to submit.

Enclosed is the Summary Statement containing the comments of the initial reviewers of your research proposal. I am also enclosing an explanation of the derivation and use of the NIH priority scores.

If you have questions concerning your application or the review process, please feel free to contact me.

Sincerely,

Edward Gardner Jr.

Edward Gardner, Jr., Ph.D.
Program Director
Regular Research Programs Section
Scientific Programs Branch
Extramural Program

2 Enclosures

cc: Institutional Officials

REPOSITORY Oak Ridge Operations
COLLECTION Records Holding area Documents 1944-94
BOX No. B-87-7 Bldg. 2714-H
FOLDER 40-11-93-81 NIH

40-1193-81

1078991

JUL 29 1981

Division of Research Grants
National Institutes of Health
Westwood Building, Room 240
5333 Westbard Avenue
Bethesda, Maryland 20205

Gentlemen:

ORNL GRANT PROPOSAL, "HUMAN CELL ASSAY OF ENVIRONMENTAL IMMUNOTOXICANTS"
(INTERAGENCY AGREEMENT DOE NO. 40-1193-81)

Enclosed for your consideration are six copies of the subject research proposal, prepared by the DOE's Oak Ridge National Laboratory (ORNL). Also enclosed are an executed Grant Certification and an executed Cost Sharing Agreement in the format of HEW Form 430.

The objective of the proposed research is to develop an in vitro assay system to test the effect of environmental agents on immunoglobulin synthesis and secretion. The proposal requests a three-year period of performance, beginning July 1, 1982. The initial twelve month funding will require $66,700 for direct costs.

Your favorable consideration of the proposal should be reflected in a funding authorization to this office. Upon its receipt, we will authorize ORNL to proceed with the research project. Monthly billings will be accomplished by Standard Form 1081 to reimburse the DOE appropriation for actual cost incurred.

ER-13:WRBibb:mac:6-0742:7-28-81

40-1193

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DOE F 1325.10
(7-79)

OFFICIAL FILE COPY

Technical questions concerning the proposal should be directed to G. D. Griffin, ORNL Principal Investigator, telephone FTS 624-2713. Administrative questions should be addressed to J. E. Carr, ORNL Work for Others Coordinator, telephone FTS 624-6002.

Sincerely,

Original Signed by
William R. Bibb

William R. Bibb, Director
Research Division

ER-13:WRB

Enclosures:
1. Proposal (6)
2. Grant Certification
3. Cost Sharing Agreement

cc w/encls:
W. P. Snyder, CC-10, ORO

cc w/o encls:
H. Postma, ORNL
J. E. Carr, ORNL
G. D. Griffin, ORNL

ER-13
Bibb:rs
WRB
DATE
7/29/81
RTG. SYMBOL
INITIALS/SIG.
DATE

1078993

COST SHARING AGREEMENT

Awarding Office:
National Institutes of Health

Application or Award Number:

Applicant:
U. S. Department of Energy
Oak Ridge Operations Office
Research Division
Post Office Box E
Oak Ridge, Tennessee 37830

Proposed Project Period:
From July 1, 1982 to June 30, 1985

ORNL RESEARCH GRANT PROPOSAL, "HUMAN CELL ASSAY OF ENVIRONMENTAL IMMUNOTOXICANTS" (INTERAGENCY AGREEMENT DOE NO. 40-1193-81)

In order to satisfy HEW cost sharing requirements, the U. S. Department of Energy agrees to bear 17.18 percent of the total cost of the above project.

Total project cost is the sum of the total allowable direct and indirect cost incurred by the recipient and sub-recipients of cost-type contractors, plus the value of any third party in-kind contributions which benefit the project. These amounts will be calculated according to the rules in the HEW grant administration regulations at 45 CFR, Part 74.

If the project period consists of more than one budget period, the agreed-upon rate will apply to the project period as a whole, including any non-competitive extensions but not necessarily to each individual budget period.

If HEW stops funding this project prior to the close of the originally agreed-upon project period, the rate will apply to the actual period of support.

If the project period is extended competitively, another cost sharing agreement will be negotiated.

William R. Bibb

William R. Bibb, Director
Research Division

7-29-81
Date

1078994

Certification Required by PHS: 1-515-40B

for

HUMAN CELL ASSAY OF ENVIRONMENTAL IMMUNOTOXICANTS

To Be Performed By

The Department of Energy's Oak Ridge National Laboratory

I certify that:

1. The Department of Energy has the statutory authority to carry out the research study set forth in the proposal for the research project with the above title.

2. The Department of Energy will be legally, financially and administratively responsible for the conduct of the research project with the above title if a research grant is awarded to Oak Ridge Operations of the Department of Energy for this purpose.

3. Dr. William R. Bibb, Director, Research Division, Oak Ridge Operations, is delegated by me to administer the grant for the Department of Energy.

R. J. Hart
R. J. Hart
Manager
Oak Ridge Operations
Department of Energy
JUL 29 1981

1078995

OAK RIDGE NATIONAL LABORATORY
OPERATED BY
UNION CARBIDE CORPORATION
NUCLEAR DIVISION

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37830

OFFICE OF THE DIRECTOR

July 28, 1981

Department of Energy
Oak Ridge Operations
Attention: Mr. J. A. Lenhard, Assistant Manager
for Energy Research and Development
Post Office Box E
Oak Ridge, Tennessee 37830

Gentlemen:

Research Grant Proposal to the NIH, DOE IAG No. 40-1193-81,
"Human Cell Assay of Environmental Immunotoxicants"

Enclosed for your review and approval is a research grant proposal being submitted to the National Institutes of Health. The proposal requests a three-year period of performance beginning July 1, 1982, and a total direct cost of $214,700. The initial twelve-month direct funding requirement is $66,700.

The objective of this research is to develop an *in vitro* assay system to test the effect of environmental agents on one parameter of immune function, immunoglobulin synthesis and secretion.

The following information is provided to assist in your review and approval of this proposal:

a. This research effort is described in the approved ORNL Institutional Plan, "Work for Others (Excluding NRC)." The personnel required for this research are included in the direct FTE's in the "Summary of Resources."

b. G. D. Griffin will serve as principal investigator and devote 45% of his time to this research. He currently devotes 100% of his time to Assessment Activities (DOE and EPA) in P. J. Walsh's group.

c. Existing space and resources are available to conduct this research without interference to ongoing DOE programs.

40-1193-81

1078996

d. This research will serve DOE related objectives in Activity HA 02 02 03 - Biomedical and Environmental Research - Systems Damage. Based upon this programmatic relation, it is requested that this research be conducted on an actual cost basis, depreciation and DOE added cost factor waived.

After your approval, please forward six copies of the proposal to the Division of Research Grants, National Institutes of Health, Room 240, Westwood Building, 5333 Westbard Avenue, Bethesda, Maryland 20205, and one copy to Dr. Edward Gardner, Jr., Program Director, Regular Research Programs Section, Scientific Programs Branch, Extramural Programs, National Institutes of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709.

If you have any questions concerning this proposal, please contact G. D. Griffin, extension 6-2713.

Sincerely yours,

Herman Postma

Herman Postma
Director

HP:GDG:jp

Enclosure

cc: J. E. Carr
G. D. Griffin
R. F. Hibbs
S. V. Kaye
W. R. Ragland
C. R. Richmond
File - RC

1078997

FORM APPROVED
OMB NO. 68-R0101

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

GRANT APPLICATION

FOLLOW INSTRUCTIONS CAREFULLY

LEAVE BLANK

TYPE	ACTIVITY	NUMBER
REVIEW GROUP		FORMERLY
COUNCIL BOARD (Month, year)		DATE RECEIVED

1. TITLE OF APPLICATION (Do not exceed 56 typewriter spaces)
Human Cell Assay of Environmental Immunctoxicants

2. RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT ___ NO x YES (If "YES," state RFA number and/or announcement title)
NIH-NIEHS-EP-81-3: Immunotoxicology of Environmental Agents

3. PRINCIPAL INVESTIGATOR PROGRAM DIRECTOR

3a. NAME (Last, first, middle)
Griffin, Guy David

3b. SOCIAL SECURITY NUMBER
[redacted]

3c. MAILING ADDRESS (Street, city, state, zip code)
Health and Safety Research Division
Oak Ridge National Laboratory
P. O. Box X, Building 4500S
Oak Ridge, TN 37830

3d. POSITION TITLE
Research Staff Scientist

3e. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
Health Effects and Epidemiology Group

3f. TELEPHONE (Area code, number and extension)
(615) 576-2713

3g. MAJOR SUBDIVISION
Health and Safety Research Division

4. HUMAN SUBJECTS, DERIVED MATERIALS OR DATA INVOLVED
[X] NO [ ] YES (If "YES," form HHS 596 required)

5. RECOMBINANT DNA RESEARCH SUBJECT TO NIH GUIDELINES
[X] NO [ ] YES

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD (This application)
From: July 1, 1982 Through: June 30, 1985

7. TOTAL DIRECT COSTS REQUESTED FOR PROJECT PERIOD (from page 5)
$ 214,700

8. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD (from page 4)
$ 66,700

9. PERFORMANCE SITES (Organizations and addresses)
Biology, and Health and Safety Research Divisions
Oak Ridge National Laboratory
Oak Ridge, TN 37830

10. INVENTIONS (Competing continuation application only)
Were any inventions conceived or reduced to practice during the course of the project?
[ ] NO [ ] YES - Previously reported
[ ] YES - Not previously reported N/A

11. APPLICANT ORGANIZATION (Name, address and congressional district)
Department of Energy
Oak Ridge Operations
Post Office Box E
Oak Ridge, TN 37830
Third Congressional District

12. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT (See instructions)
Code 60 Description Department of Energy

13. ENTITY IDENTIFICATION NUMBER

14. TYPE OF ORGANIZATION (See instructions)
[ ] Private Nonprofit
[X] Public (Specify Federal, State, Local): Federal

15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address and telephone number)
William R. Bibb, Director
Research Division
P. O. Box E
Oak Ridge, TN 37830
(615) 576-0742 FTS 626-0742

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, address and telephone number)
William R. Bibb, Director
Research Division
P. O. Box E
Oak Ridge, TN 37830
(615) 576-0742 FTS 626-0742

17. PRINCIPAL INVESTIGATOR PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.
SIGNATURE OF PERSON NAMED IN 3a (In ink. "Per" signature not acceptable): Guy D. Griffin
DATE: 7-20-81

18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)
SIGNATURE OF PERSON NAMED IN 16 (In ink. "Per" signature not acceptable): William R Bibb
DATE: 7-29-81

PHS-398
Rev. 5/80

1078998

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

ABSTRACT OF RESEARCH PLAN

LEAVE BLANK

PROJECT NUMBER

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)

Department of Energy

TITLE OF APPLICATION (Same as Item 1, page 1)

Human Cell Assay of Environmental Immunotoxicants

Name, Title and Department of all professional personnel engaged on project, beginning with Principal Investigator-Program Director

Dr. G. D. Griffin, Staff Scientist, Health and Safety Research Division
Mr. D. D. Schuresko, Staff Scientist, Health and Safety Research Division
Dr. G. D. Novelli, Group Leader, Biology Division
Dr. Alan Solomon, Professor of Medicine, Department of Medicine, University of Tennessee Memorial Research Center

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. DO NOT EXCEED THE SPACE PROVIDED.

The purpose of this research is to develop an in vitro assay for the effect of environmental agents on one aspect of immune function, immunoglobulin production by a human lymphoblastoid cell line, and to validate this assay by testing chemicals and physical agents such as radiation in this system. The nature and kinetics of immunoglobulin production in the unperturbed cell system have been well-characterized. Using to advantage the exquisite specificity of antigen-antibody recognition, and biochemical probes of protein synthesis, it will be possible to detect changes in immunoglobulin structure (including primary sequence changes and molecular assembly alterations) in response to the action of environmental agents. The techniques of flow cytometry will allow detection of alterations in percentage of cells producing immunoglobulin, percentage of cells producing immunoglobulin fragments, and/or changes in pattern of membrane-bound immunoglobulin. This cellular system could be coupled to suitable enzyme-rich tissue extracts, to detect immunotoxicity of metabolites of chemicals inert without enzymatic activation. This in vitro cell assay could serve as an initial screening system for immunotoxicological activity; chemicals active in this system could be used in whole animal tests.

LABORATORY ANIMALS INVOLVED. Identify by common names. If none, state "none"

None

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TABLE OF CONTENTS

Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 5a, 5b. Type the name of the Principal Investigator. Program Director at the top of each printed page and each continuation page.

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Other Biographical Sketches *(Not to exceed two pages for each)*	9-13
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Resources and Environment	15

SECTION 2.

Introduction *(Excess pages; revised and supplemental applications)*	--
Research Plan	
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B. Significance *(Not to exceed three pages)*	16-17
C. Progress Report Preliminary Studies *(Not to exceed eight pages)*	17-25
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E. Human Subjects, Derived Materials or Data	--
F. Laboratory Animals	--
G. Consultants	--
H. Consortium Arrangements or Formalized Collaborative Agreements	--
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SECTION 3. Appendix *(Six sets) (No page numbering necessary for Appendix)*

Number of abstracts: 3 Number of manuscripts: _____

Other items (list):

Application Receipt Record, form PHS 3830

Form HHS 596 if Item 4, page 1, is checked

1079000

PRINCIPAL INVESTIGATOR PROGRAM DIRECTOR G. D. Griffin

DETAILED BUDGET FOR FIRST 12 MONTH BUDGET PERIOD
DIRECT COSTS ONLY

FROM | THROUGH

DOLLAR AMOUNT REQUESTED (Omit cents)

PERSONNEL (Applicant organization only) (See instructions)		TIME EFFORT				
NAME	TITLE OF POSITION	%	Hours per Week	SALARY	FRINGE BENEFITS	TOTALS
Guy D. Griffin, Ph.D.	Principal Investigator	45		$ 17,000	$ 5,000	$ 22,000
Daniel D. Schuresko, M.S.	Co-Investigator	5		2,000	600	2,600
G. David Novelli, Ph.D.	Co-Investigator	10		None	None	None
Bruce A. Owen, B.S.	Technician		30	16,500	5,000	21,500
	SUBTOTALS →			$ 35,500	$10,600	$ 46,100

CONSULTANT COSTS (See instructions)		
Alan Solomon, M. D.		0
EQUIPMENT (Itemize)		0
SUPPLIES (Itemize by category)		
Tissue Culture media and chemicals	$ 1,500	
Immunological Reagents and Other Chemicals	1,500	
Glassware (Tissue Culture Plasticware, etc.)	2,000	5,000
TRAVEL — DOMESTIC		600
TRAVEL — FOREIGN		0
PATIENT CARE COSTS — INPATIENT		0
PATIENT CARE COSTS — OUTPATIENT		0
ALTERATIONS AND RENOVATIONS (Itemize by category)		0
CONTRACTUAL OR THIRD PARTY COSTS (See instructions)		0
OTHER EXPENSES (Itemize by category)		
Utility and maintenance charges for Tissue Culture Laboratory	$ 6,200	
Upgrade Electronic components of Model 4800A Cytofluograph	2,000	
Division Administration Costs (*See Justification, p. 5)	6,000	
Graphic Arts, Photography and Publication Costs	800	15,000
TOTAL DIRECT COSTS (Also enter on page 1, item 8) →		$ 66,700

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PRINCIPAL INVESTIGATOR PROGRAM DIRECTOR G. D. Griffin

BUDGET ESTIMATES FOR ALL YEARS OF SUPPORT REQUESTED
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		1st BUDGET PERIOD (from page 4)	ADDITIONAL YEARS SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL (Salary and fringe benefits.) (Applicant organization only)		$ 46,100	$ 49,800	$ 53,800		
CONSULTANT COSTS		0	0	0		
EQUIPMENT		0	0	0		
SUPPLIES		5,000	5,500	6,000		
TRAVEL	DOMESTIC	600	800	1,000		
	FOREIGN	0	0	0		
PATIENT CARE COSTS	INPATIENT	0	0	0		
	OUTPATIENT	0	0	0		
ALTERATIONS AND RENOVATIONS		0	0	0		
CONTRACTUAL OR THIRD PARTY COSTS		0	0	0		
OTHER EXPENSES		15,000	14,800	16,300		
TOTAL DIRECT COSTS		$ 66,700	$ 70,900	$ 77,100		

TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD (Also enter on page 1, item 7) → $ 214,700

JUSTIFICATION (Use continuation pages if necessary): Briefly describe the specific functions of the personnel and consultants. For all years, justify any costs for which the need may not be obvious, such as equipment, foreign travel, alterations and renovations, and contractual or third party costs. For future years justify any significant increases in any category. In addition, for COMPETING CONTINUATION applications, justify any significant increases over current level of support. If a recurring annual increase in personnel costs is anticipated, give percentage.

*ORNL uses the Uniform Cost Accounting System recommended by the Federal Cost Accounting Standards Board. This system makes use of Cost Collect Centers in which salaries of scientific and technical personnel performing similar tasks are averaged; associated costs (fringe benefits and Health and Safety Research Division Administration) are calculated as percentages (28% and 12%, respectively) of that average cost.

Indirect costs have been established at a rate of 35% through an agreement negotiated between the NIH and the DOE. Because this percentage covers only part of costs that are often included by other institutions as indirect charges and because the DOE requires recovery of full costs, certain charges (utilities and Division Administration) are listed as Direct Costs on this form (shown under "Other Expenses").

G. D. Griffin will direct the entire project and develop any new analytical methods necessary for execution of the work. D. D. Schuresko's expertise in electronics and optical systems will be required for utilization of the cytofluorograph. G. D. Novelli initiated studies of the BF cells at ORNL, and his experience and expertise will be important in developing this project. A. Solomon will provide immunological expertise and supply certain otherwise unobtainable immunological reagents. He is currently under contract with the Health and Safety Research Division as a regular consultant, and no additional consulting cost is requested. B. A. Owen, a technician, provided

1079002

JUSTIFICATION: (Continued)

all the technical assistance in obtaining the results reported in "Preliminary Studies", and is competent in all assays proposed in this application.

Salary and supply costs will be increased 8% annually.

Travel funds include one trip annually for prinicipal investigator to a national scientific meeting (e.g., Federation).

The budgetary item for the Tissue Culture Laboratory, which is essential to conduct the proposed research, covers our cost to use these facilities.

Requested electronic alterations for cytofluorograph will be necessary for optimal use of the instrument with the new 4W Argon-Ion laser which has already been purchased.

The cost of electronic alterations for the cytofluorograph is included only in the first year's budget. Thereafter, $500 annually for service and maintenance by Ortho Instruments personnel will be included in "Other Expenses". The remaining costs in this category will be increased 10% annually.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR G. D. Griffin

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Guy David Griffin	Research Staff Scientist	[redacted]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles of complete references to recent representative publications especially those most pertinent to this application. Do not exceed 2 pages.

Summer, 1962 - NSF Research Assistantship at Iowa State University
1962-64 - Two Academic Scholarships held simultaneously, Univ. of Nebr. at Omaha
1964 - American Institute of Chemists, Outstanding Chemistry Graduate
1964 - Graduated Summa cum Laude, Univ. of Nebr. at Omaha
1965-71 - Pediatrics Laboratory Supervisor under Dr. G. E. Gibbs, Univ. of Nebraska Medical Center. Studies of the biochemical defect in Cystic Fibrosis, using techniques of enzymology, cytochemistry and tissue culture.
1969 - Full Member, Sigma Xi
1971-72 - Research Fellowship Award, National Cystic Fibrosis Research Foundation
1971-72 - Research Instructor, Pediatrics, University of Nebraska Medical Center
1972-74 - American Cancer Society Postdoctoral Fellowship, with Dr. G. D. Novelli, Biology Division, ORNL - alterations in tRNA species produced during lymphocyte blastogenesis
1974-76 - USPHS (NCI) Postdoctoral Fellowship, Biology Division, ORNL, with Drs. Jacobson and Novelli - development of separation systems for biologically-derived macromolecules (tRNA's and transfer factor), development of models to study cellular immunity biochemically.
1976-78 - Research Associate, Biology Division, ORNL (with Dr. G. D. Novelli) - biochemical mechanisms of cellular immunity, particularly transfer factor and immune RNA.
1978-Present - Research Staff Scientist, Health and Safety Research Division, ORNL, (in P. J. Walsh's Group) - Development of In vitro mammalian cell systems which may be useful in assessing hazards from chemicals.

Representative Publications

Beta-Glucuronidase Activity in Skin Components of Children with Cystic Fibrosis. G. E. Gibbs and G. D. Griffin. Science 167: 993-994, 1970.

Lysosomal Enzymes of Cultured White Blood Cells in Cystic Fibrosis. G. D. Griffin and G. E. Gibbs. Proc. Soc. Exp. Biol. Med. 137: 438-442, 1971.

Transfer RNA Species in Human Lymphocytes Stimulated by Mitogens and in Leukemic Cells. G. D. Griffin, W-K Yang and G. D. Novelli. Arch. Biochem. 176: 187-196, 1976.

Separation of Transfer Ribonucleic Acids on Polystyrene Anion Exchangers. R. P. Singhal, G. D. Griffin, and G. D. Novelli. Biochem. 15: 5083-5087, 1976.

(Continued)

1079004

[redacted]

BIOGRAPHICAL SKETCH - G. D. Griffin (Continued)

Optimization of Phenol Extraction Procedures for Preparation of RNA from Mammalian Lymphoid Organs. G. D. Griffin, H. G. Sellin and G. D. Novelli. Anal. Biochem. 87: 506-520, 1978.

Chemical Cytotoxicity - A Cancer Promoter. G. D. Griffin, T. D. Jones and P. J. Walsh. Polynuclear Aromatic Hydrocarbons - Third International Symposium, Ed. by P. W. Jones and P. Leber, Ann Arbor Science Pub., 723-732, 1979.

Reproductive Survival and Macromolecular Synthesis in Cultured Mammalian Cells Exposed to Single Metabolites and Mixtures of Metabolites of Benzo(a)pyrene. G. D. Griffin, C. S. Dudney, P. S. Furcinitti, T. D. Jones and P. J. Walsh. Polynuclear Aromatic Hydrocarbons - Fourth International Symposium, Ed. by A. Bjorseth and A. J. Dennis, Battelle Press, 287-298, 1980.

Biochemical Studies of Immune RNA Using a Cell-Mediated Cytotoxicity Assay. G. D. Griffin, H. G. Sellin and G. D. Novelli. Cancer Biochem. Biophys. 4: 123-131, 1980.

Assessment of Potential Health Effects of Dielectric Gases. G. D. Griffin, C. E. Easterly and P. J. Walsh. Gaseous Dielectric II. Ed. by L. G. Christophorou, Pergamon Press, 439-449, 1980.

Empirical Observations in Support of Carcinogenic Promotion as a Tool for Screening and Regulation of Toxic Agents. T. D. Jones, G. D. Griffin, C. S. Dudney and P. J. Walsh. Polynuclear Aromatic Hydrocarbons - Fifth International Symposium, October 28-30, 1980. Battelle Columbus Laboratories, In Press.

In Vitro Sensitization of Human Lymphocytes to a Myeloma Cell-Related Antigen. M. E. Whitson, G. D. Griffin, G. D. Novelli and A. Solomon. Cell. Immunol. 60: 489-497, 1981.

Aryl Hydrocarbon Mono-Oxygenase Activity in Human Lymphocytes. G. D. Griffin and D. D. Schuresko. ORNL/TM-7721, 1981, 20 pgs.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR G. D. Griffin

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME		BIRTHDATE (Mo., Day, Yr.)
Daniel D. Schuresko	Research Staff Scientist	[redacted]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

Summers 1964-69 - Draftsman, Lehigh Design Co., Dravosburg, PA

[redacted]

Summer 1970 - Research Assistant in Optics Research Laboratory, Penn State University
1970-72 - Teaching Assistant, Physics Department, Cornell University
1972-77 - Research Assistant, School of Applied and Engineering Physics, Cornell Univ.
1977-80 - Research Associate and Staff Member, Chemical Technology Division, ORNL
1980-Present - Staff Member, Health and Safety Research Division, ORNL
1980 - Industrial Research and Development IR-100 Award for the Portable Fluorescence Spotter

Representative Publications

Carboxylation Kinetics of Hemoglobin and Myoglobin: Linear Transient Response to Step Perturbation by Laser Photolysis. Biophys. J. Vol. 24: #1: 382, 1978.

A Portable Fluorometric Monitor to Detect PNA Contamination of Work Area Surfaces. Proceedings of the Symposium on Assessing the Industrial Hygiene Monitoring Needs for the Coal Conversion and Oil Shale Industries, Brookhaven National Laboratory, 1978. BNL 51002.

A Portable Fluorometric Monitor to Detect Polynuclear Aromatic Hydrocarbon Contamination of Work Area Surfaces. Proceedings of the 1979 Symposium on Instrumentation and Control for Fossil Energy Processes, Denver, Colorado, 1980. ANL 79-62, Argonne National Laboratory.

New Instruments for Plant Area and Personnel Monitoring. D. D. Schuresko, R. B. Gammage, A. R. Hawthorne, and T. Vo-Dinh. Proceedings of the Third Miami International Conference on Alternative Sources, 1980 (To be Published.)

Portable Fluorometric Spotter for Detection of Surface Contamination by Polynuclear Aromatic Hydrocarbon Compounds. Fifth International Symposium on Polynuclear Aromatic Hydrocarbons, Battelle Columbus Laboratories, 1980 (In Press).

Portable Fluorometric Monitor for Detection of Surface Contamination by Polynuclear Aromatic Compounds, Anal. Chem. 52: 371, 1980.

1079006

PRINCIPAL INVESTIGATOR PROGRAM DIRECTOR G. D. Griffin

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
G(uerino) David Novelli	Group Leader in Enzymology	[redacted]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

Summer 1940 - Sanitary Engineer Fellowship - W.K. Kellogg Foundation, Battle Creek, MI
1942-44 - Univ. of Calif., Scripps Institute of Oceanography - worked toward Ph.D. in marine microbiology with minor in marine biochemistry - complete except for dissertation.
1944-46 - Army of the United States - Master Sergeant, 4th Service Medical Command, Fort McPherson, Atlanta, GA - medical diagnostic microbiology laboratory.
1949-53 - Biochemical Research Laboratories at the Massachusetts General Hospital, assistant to associate biochemist under Dr. Fritz Lipmann.
1952-Present - American Association for the Advancement of Science (Fellow)
1953-56 - Associate Professor of Microbiology, Western Reserve University School of Medicine
1956-Present - Principal Biochemist and Group Leader in Enzymology in the Biology Division, ORNL
1960-63 - Membership Committee, American Society of Biological Chemists
1960-Present - Research Professor of Biochemistry, University of Georgia
1961-62 - Chairman, Atomic Energy Commission Committee on DNA, RNA and Proteins
1961-65 - Physiological Chemistry Study Section, U. S. Public Health Service
1964-68 - Radiation Study Section, U. S. Public Health Service
1965-69 - Chairman, Advisory Committee on Research on the Etiology of Cancer, American Cancer Society
1965-73 - Director, Macromolecular Separations Technology Program, ORNL
1967-Present - Professor of Biomedical Sciences, University of Tennessee Oak Ridge Graduate School of Biomedical Sciences
1969-70 - Foundation for Microbiology National Lecturer, American Society for Microbiology
1969-70 - Scholar in Cancer Research, American Cancer Society
1969-73 - Physiology Chemistry Study Section, U. S. Public Health Service
1970-74 - Chairman, The Advisory Committee for Personnel for Research, American Cancer Society
December 1971 - Participant in The National Cancer Program Planning Conference
1971-Present - Adjunct Professor of Chemistry, University of California, San Diego
1972-73 - Ad Hoc Review Committee of Research Programs in Basic Science for the Veterans Administration
1974-75 - Member Advisory Board for The Enzyme Engineering Conference of the Engineering Foundation
1975-Present - Member Scientific Advisory Committee, University of Rochester Cancer Center
1975-Present - Member International Advisory Board Cancer Biochemistry Biophysics
1975-79 - Member Drug Development Contract Review Committee for the Division of Cancer Treatment, National Cancer Institute

(Continued)

BIOGRAPHICAL SKETCH - G. D. Novelli (Continued)

Publications

More than 200 in professional journals and articles in books and symposium reports. Many oral presentations at national and international meetings. Research interests include enzymology of biosynthetic reactions, molecular basis for genetic control of protein structure and synthesis, structure and function of transfer RNA, carcinogenesis, antibody synthesis, microbial metabolism, fermentation technology, enzyme engineering, separation and purification of tumor specific transfer factor, and recombinant DNA technology.

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BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Alan Solomon	Professor of Medicine	[redacted]

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
[redacted]			

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

[redacted]

1960-62 - Clinical Associate, Metabolism Service, NCI, NIH under Drs. J. L. Fahey & N. I. Berlin
1960-62 - Senior Assistant Surgeon, USPHS
1961 - Dr. Daniel Stats Memorial Award, Editorial Board, J. of The Mount Sinai Hospital
1962-Present - Senior Surgeon, Inactive Reserve, USPHS
1963 - American Association for Cancer Research
1963 - American Medical Association
1963-65 - Special Fellowship, National Institute of Arthritis & Metabolic Diseases, NIH, USPHS
1963-65 - Research Assistant in Hematology (Dr. L. R. Wasserman), The Mt. Sinai Hospital, New York
1963-65 - Guest Investigator & Assistant Physician (Dr. H. G. Kunkel), The Rockefeller University, New York
1964 - American Society of Hematology
1964 - Diplomate, American Board of Internal Medicine
1965-66 - Assistant Attending Hematologist, The Mt. Sinai Hospital, New York
1965-66 - Associate Physician and Guest Investigator, The Rockefeller University, N.Y.
1966-69 - Associate Research Professor, The University of Tennessee Memorial Research Center and Hospital (MRCH)
1966-85 - USPHS Research Grant CA-10056, Principal Investigator
1967-73 - American Cancer Society Research Grant T-420, Principal Investigator
1967-73 - National Science Foundation Research Grant GB-6036, Principal Investigator
1967 - Central Society for Clinical Research
1968 - American Association of Immunologists
1968 - Tennessee Medical Association
1968 - Sigma Xi
1968 - American College of Physicians (Fellow)
1968 - Southern Society for Clinical Investigation
1968-72 - Research Career Development Award CA 21,229, NCI, NIH, USPHS
1968-Present - Institutional Research Grant Committee, American Cancer Society, University of Tennessee - Chairman
1970 - Second Prize, Henry L. Moses Award, Montefiore Hospital, New York
1971 - Physician's Recognition Award, American Medical Association
1970-78 - Research Professor, The University of Tennessee MRCH

BIOGRAPHICAL SKETCH - Alan Solomon, M.D. (Continued)

1971-Present - Subcommittee on Nomenclature of Human Immunoglobulins, International Union of Immunologic Societies
1971 - American Society for Clinical Investigation
1972 - Third Prize, Henry L. Moses Award, Montefiore Hospital, New York
1972-Present - Clinical Professor, Department of Internal Medicine, Clinical Education Center, U.T. Center for Health Sciences/Knoxville
1974-77 - USPHS Research Grant CA-15173, Co-Principal Investigator
1976-Present - Consultant, Human Cancer Diagnostics and Therapeutics Unit of the Cancer and Toxicology Program, Biology Division, ORNL
1976 - Physician's Recognition Award, American Medical Association
1977 - American Association for Cancer Education
1977 - American Society of Clinical Oncology
1977-Present - Consultant, Biomedical and Environmental Research Division, Argonne National Laboratory
1978-Present - Member, Advisory Panel, The American Board of Medical Laboratory Immunology
1978-79 - Consultant, Clinical Cancer Program Project Review Committee, NCI, NIH
1978-80 - Professor of Medical Biology, Department of Medical Biology, MRCH, U. T. Center for Health Sciences/Knoxville
1979-83 - Member, Clinical Cancer Program Project Review Committee, NCI, NIH
1979 - Physician's Recognition Award, American Medical Association
1980 - International Society of Hematology
1980-Present - Professor of Medicine, Department of Medicine, U.T. Center for Health Sciences/Knoxville

Representative Publications

More than 115 publications and abstracts, plus numerous presentations at national and international scientific meetings, as an invited lecturer, as part of the University of Tennessee teaching activity responsibilities, at area hospitals, before local civic organizations, and in radio and television appearances. Interests include various aspects of humoral immunity, immunoglobulin structure and function, hematologic cancers and other hematologic disorders, humoral and cellular immunity in cancer.

1079010

OTHER SUPPORT

(USE CONTINUATION PAGES IF NECESSARY)

For each of the professionals named on page 2, list, in three separate groups: (1) active support; (2) applications pending review and/or funding; (3) applications planned or being prepared for submission. Include all Federal, non-Federal, and institutional grant and contract support. If none, state "NONE." For each item give the source of support, identifying number, project title, name of principal investigator/program director, time or percent of effort on the project by professional named, annual direct costs, and entire period of support. (If part of a larger project, provide the titles of both the parent grant and the subproject and give the annual direct costs for each.) Briefly describe the contents of each item listed. If any of these overlap, duplicate, or are being replaced or supplemented by the present application, justify and delineate the nature and extent of the scientific and budgetary overlaps or boundaries.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: G. D. Griffin

(1) ACTIVE SUPPORT: EPA #-20074020 - "Risk Assessment of Synthetic Fuels"; principal investigator - P. J. Walsh; percent effort - 100%; annual direct costs - $325,000. Contract ends April 1982.

(2)APPLICATIONS PENDING: DOE FTP - "Aryl Hydrocarbon Mono-Oxygenase Induction: A Measure of PNA Exposure." HA 010201; D. D. Schuresko, G. D. Griffin, P. F. Mullaney and G. D. Novelli; percent effort - 30%; annual direct costs - $148,000. Three years requested.

(3)APPLICATIONS PLANNED: None.

D. D. Schuresko

(1)ACTIVE SUPPORT: DOE FTP - "Dosimetry for New Energy System Pollutants"; Activity #001707, principal investigator - R. B. Gammage; percent effort - 100%; annual direct costs - $260,000. Funding through FY 81.

(2)APPLICATIONS PENDING: DOE FTP - "Aryl Hydrocarbon Mono-Oxygenase Induction: A Measure of PNA Exposure." HA 020102; D. D. Schuresko, G. D. Griffin, P. F. Mullaney and G. D. Novelli; percent effort - 50%; annual direct costs - $148,000. Three years requested.

(3)APPLICATIONS PLANNED: None.

G. D. Novelli

(1)ACTIVE SUPPORT: DOE FTP - "Bioprocessing for Energy Production"; Activity # HB-0100000; principal investigator - G. D. Novelli; percent effort - 100%; annual direct costs - $104,000. Funding through FY 81.

(2)APPLICATIONS PENDING: DOE FTP - "Aryl Hydrocarbon Mono-Oxygenase Induction: A Measure of PNA Exposure"; HA020102; D. D. Schuresko et al.; percent effort - 10%; annual direct costs - $148,000.

(3)APPLICATIONS PLANNED: None.

RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Use "other" to describe facilities at other performance sites listed in Item 9, page 1, and at sites for field studies. Using continuation pages if necessary, include a description of the nature of any collaboration with other organizations and provide further information in the RESEARCH PLAN.

☒ Laboratory: Complete tissue culture laboratory (in G. D. Novelli's laboratory facilities) of 477 sq. ft. with HEPA-filtered air supply, 2 BioHazard Hoods, 2 CO_2 Incubators, Water Still, Autoclave, Liquid Nitrogen Cell Storage Tanks, Refrigerators and Kelvinator Freezer. Access to other general biochemical laboratories under Dr. Novelli's supervision, for certain other procedures, will be made available.

☐ Clinical:

☐ Animal:

☐ Computer:

☐ Office:

☐ Other (_____):

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location, and pertinent capabilities of each. Farrand Mark II Spectrofluorometer; Leitz Laborlux Fluorescence Microscope; Model 4800 A Cytofluorograph, with Updated Argon-Ion Laser of 4W Total power (for maximum potential, this instrument requires replacement of certain outmoded electronic components--see page 4); LKB Electrophoresis and Isoelectric Focusing Apparatus; LKB Refrigerated Fraction Collectors (All items currently in Dr. Novelli's laboratories, Biology Division, ORNL).

ADDITIONAL INFORMATION: Provide any other information describing the environment for the project. Identify support services such as consultants, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.
Dr. Alan Solomon, under a current consultant contract with the Health and Safety Research Division, will provide immunological expertise to all aspects of this project. Through his laboratory, certain immunological reagents (such as the idiotypic antisera and antisera against free κ light chain), not commercially obtainable, will be made available. The electronic upgrading of the Cytofluorograph will be carried out by Mr. Schuresko in conjunction with the Instrumentation and Controls Division of ORNL.

1019012

Griffin, G. [redacted]

A. Specific Aims

We propose to investigate the effects of environmental agents, both chemical and physical, on one well-established manifestation of immune function, immunoglobulin production. A human lymphoblastoid cell line (designated BF) which produces a single specific type (monoclonal) of immunoglobulin G (IgG), and in which production and secretion of this IgG has been well-characterized, will be used as the basis for this in vitro immune function assay. The effects of environmental agents on IgG synthesis and secretion by BF cells will be studied; measurable parameters include induced structural alterations in the IgG product, alterations in percentage of total cellular population synthesizing IgG, and alterations in quantity of IgG secreted per cell. Studies will be performed to document the capacity of BF cell enzymes to metabolize certain environmental chemicals of interest; if such enzymes are lacking, liver microsomal fractions, or cells derived from various organs will be used in experiments aimed at developing a system analogous to cell- or homogenate-mediated mutagenesis assays. Using biochemical techniques, the molecular basis for alterations in IgG production in response to environmental agents will be investigated.

B. Significance

The importance of humoral immunity in maintaining the health and integrity of the human organism is well-appreciated. The influence of environmental pollutants on the functional ability of those cells responsible for immunoglobulin synthesis has not been extensively investigated in man, however. As Adkinson (1) points, out, environmental agents can directly damage the functionality of the immune system, resulting in a state of immunodeficiency. Alternatively, pathological effects on various organ systems (such as occur in auto-immune disease states) may result from alteration of the functional ability of the immune system.

Some experimental evidence of altered humoral immunity following exposure to certain physical and chemical agents has been obtained. Vos and co-workers (2) have found that 2,3,7,8-tetrachlorodibenzo-p-dioxin, a contaminant of herbicide manufacture, slightly suppressed guinea pig humoral immunity. Thomas et al (3) have shown that exposure to cigarette smoke can alter humoral immune functions in mice. They found that the primary antibody response, following antigen challenge, was abolished in lung lymphocytes, and significantly reduced in duration and magnitude in spleen and lymph nodes of mice exposed for 26 wks to cigarette smoke. Zarkower (4) observed progressive decreases in overall antibody-forming ability of mice exposed to carbon particles alone or particles plus SO_2. The concentration of SO_2 and carbon (2 ppm and 500 $\mu g/m^3$, respectively) were designed to reflect "real life" pollution situations. Greatest effects were seen at maximum exposure duration (192 days) and involved decreases in numbers of antibody-forming cells in the spleen and decreases in hemagglutination titers in serum.

Metallic compounds have also been implicated in immune alteration. Newborn mice, when they and their dams were fed Zn-deficient diets for 4 wks following birth, showed aberrations in levels of immunoglobulins G, M and A (5). Graham et al (6) observed reduction of the primary humoral immune response of mouse spleen cells, when animals were exposed to certain concentrations of $NiCl_2$ or $CdCl_2$ by inhalation. Intramuscular injection of $NiCl_2$ or $NiSO_4$ also resulted in suppression of humoral immune response, although $CdCl_2$ and $CrCl_3$ were without effect. Physical agents such as radiation have also been found to affect humoral immunity. Balish et al (7), in studying 3 men who were accidently irradiated with X-rays (total body dose varied from 100-200 R to 500-600 R) found decreases below control levels in IgG in plasma by 20-30 days post-irradiation. Even after $1\frac{1}{2}$ yrs, fluctuation in IgG levels in these individuals was far beyond normal variation.

1079013

In these in vivo studies, the primary and secondary effect of chemicals or physical agents could not be differentiated and thus other investigators have utilized in vitro assays. If such in vitro systems could be shown to mimic or parallel in vivo immune responses, their usefulness for extrapolations to in vivo situations would be enhanced. In this respect, immunoglobulin production by lymphoblastoid cell lines may be an exceptionally good model. Fahey et al (8) note the similarities to the in vivo immune response to an antigen and state "lymphoid cells in culture mimic the normal immune response in passing through lag, proliferative and restricted (or stationary) phases of growth and immunoglobulin synthesis." Chemical treatment has been shown to perturb immunoglobulin production in certain cellular systems. Scharff and his coworkers (9, 10, 11) have investigated the effects of various mutagens on immunoglobulin production in mouse myeloma cells (malignant plasma cells). These workers found that treatment of such cells with nitrosoguanidine, melphalan, or ICR 191, an acridine half-mustard, could produce variant clones of cells which exhibited a variety of changes in immunoglobulin compared to control cells. Not all potent mutagens produced such effects, however, since ethyl methane sulfonate was ineffective in increasing the frequency of variants. The immunoglobulin changes included: loss of ability to synthesize the heavy chain portion of immunoglobulin, but retention of light chain production; loss of ability to synthesize any immunoglobulin, complete or fragmented; production of heavy chains that were of a different size (both smaller and larger), and which were of a different structure from native immunoglobulin; secretion of defectively assembled immunoglobulin (i.e., only one heavy and light chain per molecule); and defective glycosylation of immunoglobulin.

Since immunoglobulin production is an essential part of humoral immunity, a cellular system which could be used as an in vitro model for immunoglobulin production would be of importance in screening tests to study the effect of environmental agents upon humoral immunity. We have begun such studies employing the established human lymphoblastoid cell line BF which produces a monoclonal IgG protein. Use of this human cell line to assay effects of various chemicals and other agents on IgG production could provide a valuable initial step in a tiered approach to screening chemicals for immunotoxicological properties. Agents active in this human system could be referred to other lymphoblastoid lines to generalize any results, or to animal testing for further investigation of their immunological effects. BF cells apparently do not show the variability in IgG structural features seen in Scharff's system (frequent spontaneous generation of IgG variants). The unique attributes of the BF cells will thus permit future studies at the gene level of how such agents alter IgG production, and whether such effects are reversible or preventable. This understanding could aid in therapies to alleviate the deleterious effects of such agents.

C. Preliminary Studies

The basis of the proposed immunotoxicological assay is an established, IgG-synthesizing human lymphoblastoid cell line (designated BF). This cell line, originally derived from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes of a 70 yr. old healthy Caucasian male, was provided to our laboratory in 1975 by Dr. Dimitri Viza. The cells, when examined by light and electron microscopy, are morphologically heterogeneous, with features characteristic for plasma cells and mature and immature (i.e. blast cells) lymphocytes (Attached Figs. 1 and 2). This heterogeneity is also seen in clones derived from BF cells, and thus is probably indicative of a limited differentiation ability of these cells, rather than a communal mixture of cell types arising from initial isolation of the cell line. Morphological heterogeneity is characteristic of previously described human lymphoblastoid cell lines (8).

BF cells do not: form rosettes with sheep red blood cells (a T cell marker), contain receptors for complement (a B cell marker), or have phagocytic ability. Alter-

natively, the cells have the capacity to synthesize immunoglobulin as evidenced by populations of cells which resemble B lymphocytes and have cell-surface and cytoplasmic immunoglobulin (Attached Fig. 3). The amount of IgG secreted into culture medium by the BF cells/24 hrs. is ~0.5 - 1.5 μg per 10^6 cells. Of five IgG-producing lymphoblastoid lines tested, BF was found to secrete the most IgG (Dr. S. Litwin, personal communication). The cells contain EBNA (Epstein-Barr Nuclear Antigen) but are not producing EB virus (Dr. W. Henle, personal communication). The monoclonal nature of the BF cells is evidenced by the monoclonal nature of the cell surface and cytoplasmic immunoglobulin identified as IgG with κ-type light chains (IgGκ). Further, the monoclonal nature of the immunoglobulin product has also been demonstrated by us through studies of the protein secreted by the BF cells. This material has been isolated and has been shown to possess the following characteristics: heavy chain, gamma (γ) subclass γ1, GM allotype Gm(1); light chain, kappa (κ), V region subgroup II, κm allotype κm(3). (Allotype specificity performed by Dr. Arthur G. Steinberg). In addition to the intact IgG protein, the BF cells also synthesize and secrete a free monoclonal light chain (κII) that appears identical to the light chain contained in the complete molecule. The strongest present evidence of the human nature of the BF cell line is the fact it is producing human IgG. The morphologic and chemical characteristics of the BF cells indicate that they are of human origin and have features of a spectrum of B cells ranging from immature to mature lymphocytes and plasma cells.

A most significant feature of IgG secretion by these cells is that only one specific type of IgG has been detected (i.e. monoclonal antibody), suggesting that all cells synthesizing IgG are descendents of one original cell making this type of IgG. Since BF cells produce only a single IgG molecule, it is possible to prepare immunological reagents very specific to this molecular structure, and by using these reagents, to detect small alterations in structure of the IgG. Because BF cells are differentiated to IgG-synthesizing cells, complex rearrangements in IgG genes (that occur preliminary to IgG production in progenitors of the plasma cell lineage and which affect IgG structure and possibly specificity) are not expected in BF cells. Environmental agents interfering with processes controlling differentiation from a pluripotent stem cell to a plasma cell producing a specific antibody will therefore not be monitored by the BF cell system.

BF cells actively proliferate in several types of cell culture media, and are routinely grown in our laboratory in RPMI 1640, supplemented with 10% fetal calf serum and antibiotics (the concentration of fetal calf serum can be lowered to 2.5% without severely inhibiting growth or affecting rates of IgG synthesis.) These cells do not require trypsinization to remove them from glass or plastic. Under optimum conditions, the doubling time of the BF cells is ~22-24 hrs. They are apparently immortal and can be cryogenically preserved and recovered without significant loss in viability. BF cell innocula (of at least 1 x 10^6 cells) have been administered to nude mice by a variety of routes (including intraperitoneally and intraveneously), and after 7 months, no evidence of tumor growth has appeared (personal communication, James Otten, Dr. Raymond Tennant's laboratory).

BF cells can be cloned in soft agar with efficiencies of up to 50%, but only when a feeder cell layer, such as human foreskin fibroblasts is used. They can also be cloned by aliquoting single cell dilutions into microtiter plate wells, if "conditioned" medium from exponentially growing BF cultures is added. The characteristics of BF cells and their secretion product are summarized in Table I.

The IgG product secreted by BF cells has been isolated and purified using an anti-IgG affinity column. A $F(ab')_2$ product prepared by pepsin digest of the BF IgG protein was used to prepare an anti-idiotypic antiserum in rabbits (Dr. Alan Solomon's laboratory). This antiserum provides a specific probe to investigate changes induced in the variable (antigen-binding) portion of the IgG molecule and, together with commer-

cial anti-human IgG antisera (which have specificity for the γ heavy chain constant regions), provide a means of detecting serologically structural alterations in the IgG molecule.

The characteristics and variation of IgG secretion by BF cells have been intensively studied in our laboratory over the past year under temporary support provided by the Oak Ridge National Laboratories' Exploratory Studies program. The original purpose was to test the feasibility of this cell system as a human cell mutagenesis system (using IgG as a mutation marker) to assay mutagenicity of coal-derived chemicals. The results of this study relevant to the present application will be briefly summarized.

Characterization of the pattern of synthesis and secretion of IgG by the BF cell line was carried out. An apparent variability in IgG secretion was a reflection of recurring changes in IgG production related to the growth cycle of these cells. With daily complete medium changes, cultures grow to a density of $\sim 2 \times 10^6$ cells/ml and then enter a growth plateau (see Graph 1). During this growth cycle, secreted IgG increases to a maximum of 0.3 - 0.4 μg/ml and then declines, this decline occurring as the cells reach a density of $1.5 - 2.0 \times 10^6$/ml. (Graph 2). The percentage of IgG-producing cells (determined by cytoplasmic staining) shows a similar pattern, usually declining as cells enter plateau phase (Graph 2). If IgG secretion is calculated on a per cell basis (Graph 3), it is apparent that IgG secretion/cell diminishes even before the culture enters a growth plateau. Similar patterns of immunoglobulin synthesis have been reported in other lymphoblastoid lines (8, 12, 13). Quantitative assay of intracellular IgG, after cell lysis, confirmed cytoplasmic staining results, in that the actual amount of IgG per cell (averaged over a large population of cells) decreased to approximately half its initial value as cells entered growth plateau (Graph 4). Graph 4 also demonstrates that the major portion of IgG synthesized by BF cells is secreted. These results have been repeated multiple times, with different passages of BF cells, and suggest a remarkable stability for this phenomenon.

The recurring variation in IgG secretion seen as the cells cycle through exponential and plateau phase growth is unlikely to be due to exhaustion of some required precursor for IgG in the culture medium or accumulation of toxic or inhibitory products, since complete medium changes are made daily. Alternatively, this variation may result from varying expression of IgG production by different cell populations in these cultures (e.g. lymphocytes and plasma cells).

To determine the inherent variability of <u>individual</u> BF cells (e.g. would a non-producing clone eventually convert to a certain percentage of IgG-producing cells, or vice versa?), we studied the growth rate and IgG production of various clones of BF cells. We found that although there were large <u>quantitative</u> differences in IgG secretion, the percentage of cells containing IgG was similar for all clones examined (Graph 5). Furthermore, the results indicate the exquisite sensitivity of the immunofluorescent assay for intracellular IgG, in that cultures producing only small amounts of IgG (i.e. clone 25) still show a large percentage of IgG synthesizing cells. This suggests that the decreases in percentage of IgG-positive cells seen in plateau phase BF cultures may represent actual cessation of IgG synthesis in certain cells. No clones could be found that were negative for IgG production. These results suggest considerable variability in regard to quantity of IgG production among the BF cells, but that almost all BF cells are capable of such production. Therefore, cell heterogeneity seems unlikely as an explanation for variation in IgG production.

Possible explanations for the alterations in IgG production during growth cycle include: (1) IgG production occurs only during certain phases of the cell cycle (8, 13); when BF cells enter plateau phase, they do not (or only slowly) traverse the cell cycle; (2) IgG production acts as a feedback repressor of IgG synthesis, so that when levels of IgG intra- or extracellularly reach a certain level, IgG synthesis and/or

secretion is inhibited. We have only begun to investigate these possibilities. Nonetheless, our data indicate the potential usefulness of the BF cell system to investigate external perturbations of IgG production.

Experiments were also undertaken to investigate the effect of various chemical mutagens on IgG production by BF cells. The procedure for treatment of the BF cells provided a time period to permit recovery from the toxic effect of the added chemical during which the cell count and viability was monitored daily. Following recovery of viability, alterations in IgG production were monitored over at least one complete growth cycle.

The first chemical studied was the phenylalanine mustard, melphalan, a cancer chemotherapeutic agent which is a known animal carcinogen and has been suspected to be a human mutagen/carcinogen (14). The amount of drug chosen was based on that estimated to approximate human therapeutic doses. Following a significant cytotoxic effect, the treated BF cells eventually recovered full viability, with the following changes in properties: (a) a significant, reproducible, and apparently stable change in population doubling time of cells treated with 4×10^{-7}M melphalan, from approximately 24 hr to 72 hr. (Graph 6); (b) a quantitative decrease in IgG production, whether as measured by secreted IgG (μg/ml or μg/cell) or by percentage of cells containing cytoplasmic IgG (Graph 7).

The decrease in secreted IgG from melphalan-treated BF cells was readily distinguishable quantitatively, from untreated cells (varying from a minimum of one-half the value of control cultures to a maximum of one-eighth). Furthermore, the characteristic fluctuation in IgG production with cell growth was not seen in these altered cells.

Melphalan-treated cells were cloned in microtiter wells, to determine whether non-IgG-producing cells were generated by melphalan treatment. Results of this experiment indicated that all clones studied were capable of IgG production (as detected by positive cytoplasmic fluorescence) but that such clones were producing less IgG than untreated cells. Scharff (personal communication) has found a similar phenomenon in his mouse myeloma cell system. It thus seems unlikely that "nonproducing" cells, in the sense of absolute inability to synthesize IgG, were selected by melphalan treatment.

Immunological analysis of IgG produced by melphalan-treated cells did not reveal altered ("mutated") IgG molecules, as detected by precipitin reactions with a variety of anti-IgG antisera, including the anti-idiotype antisera.

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a commonly used mutagen for <u>in vitro</u> assays, was also used to treat the BF cells. Doses of MNNG of 0.2 μg/ml produced initial decreases in IgG secretion comparable to that found in the case of melphalan, while lower doses did not consistently alter IgG production from control levels. When MNNG-treated BF cells were monitored over a complete growth cycle, a lengthened population doubling time was noted (e.g. $\sim$ 72 hrs), as was also seen with melphalan-treated cells. IgG production eventually recovered to approximately 1/2 - 3/4 levels in control cultures.

The results attained to date suggest that alteration of IgG production by chemical agents does occur in BF cells, and, in fact, that this cellular function responds with high sensitivity to chemical insults. Both melphalan and MNNG are direct acting chemical agents, i.e., they do not require metabolic/enzymatic activation to covalently bind to cellular macromolecules. As yet, we have not established whether the BF cells have the requisite enzymes to metabolize chemicals (such as polynuclear aromatic hydrocarbons) to reactive metabolites. Preliminary studies with benzo(a)pyrene [B(a)P] have indicated that BF cells do not have constitutive aryl hydrocarbon hydroxylase activity, and that treatment with an appropriate inducer [B(a)P] does not result in significant enzyme activity.

1079017

[redacted]

Abstracts Pertaining to Preliminary Studies.

1. "Immunoglobulin Production as a Marker for Mutagenesis: Comparison of Chemical Activity in Lymphoid Cells of Human and Mouse Origin," G. D. Griffin, B. A. Owen G. D. Novelli, A. Solomon and P. J. Walsh. Abstract in Organ and Species Specificity in Chemical Carcinogenesis, sponsored by EPA and NCI. March 2-4, 1981.

2. "Immunoglobulin Production in a Human Lymphoblastoid Cell Line: Alteration by Melphalan, A Cancer Chemotherapy Agent." G. D. Griffin, B. A. Owen, C. Atchley, A. Solomon, P. J. Walsh and G. D. Novelli. Abstract Cancer Res. 22: 108, 1981.

3. "Control of Synthesis and Excretion of Immunoglobulin by a Human Lymphoblastoid Cell Line." G. Griffin, B. Owen, A. Lynch, C. Atchley, P. Walsh, A. Solomon, and G. D. Novelli. Abstract. Fed. Proc. 40: 1702, 1981.

Table I

Biological and Chemical Properties of
Human Lymphoblastoid Cell Line BF.

Morphology
- Light and Electron Microscopy: Lymphoblasts, lymphocytes, plasma cells

Cell Markers
- Sheep RBC Rosettes - Negative
- EAC Rosettes - Negative
- Cell Membrane Immunoglobulin - +: γ, κ; Negative: α, μ, δ, ε, λ.
- Cytoplasmic Immunoglobulin - +: γ, κ; Negative: α, μ, δ, ε, λ.
- Nonphagocytic
- EBNA - +
- EB Virus Production - Negative
- Tumorigenicity in Nude Mouse - Negative

Growth Rate
- Doubling Time ~24 hrs.

Immunoglobulin Products
- IgG - heavy chain γ, subclass $\gamma 1$, Gm allotype Gm (1)
- κ Light Chain [κII - κm(3)]

Unique Features
- Human Cell Line
- Monoclonal IgG Products (rare light chain type)
- Growth Rate
- High Cloning Efficiency in Agar
- Stability of IgG Production
- Apparently Immortal

1079018

Morphology of BF Cells

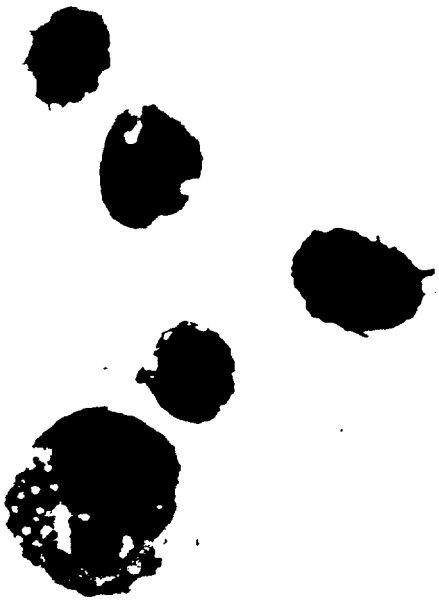

Fig. 1 Photomicrograph (250 x) stained with Wright's stain

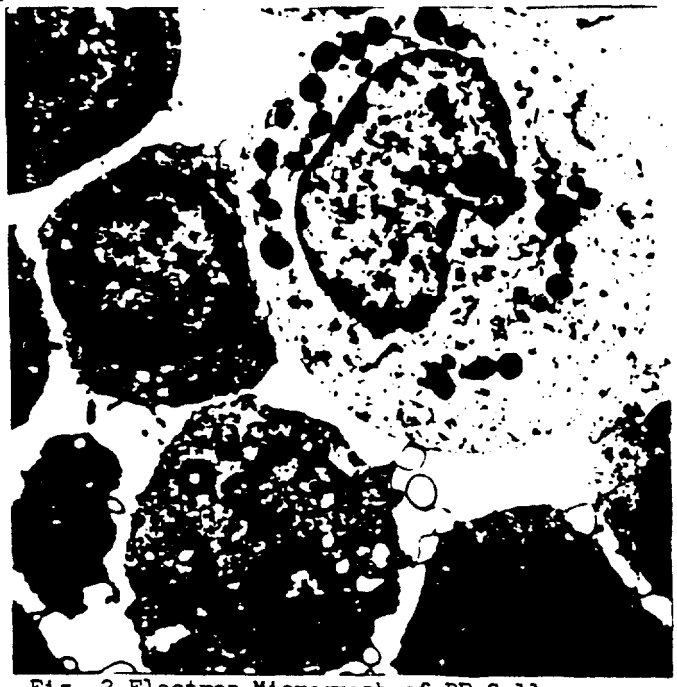

Fig. 2 Electron Micrograph of BF Cells (5,000 X)

Immunohistochemistry of BF Cells

Fig. 3A. Fluorescence Micrograph of Fixed cells stained with FITC-conjugated antihuman IgG (γ) antisera (450 x)

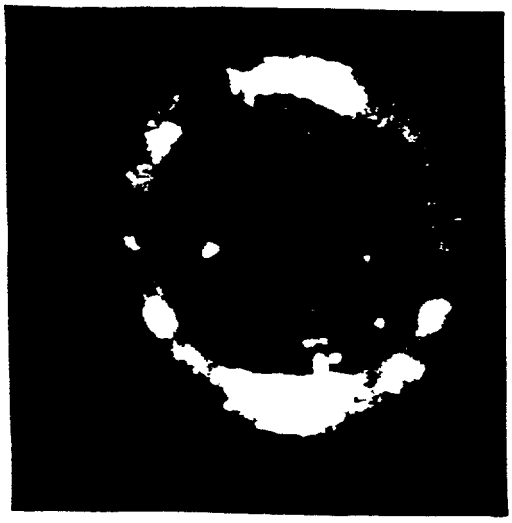

Fig. 3B. Fixed cells stained with FITC-conjugated antihuman kappa antisera (450 x)

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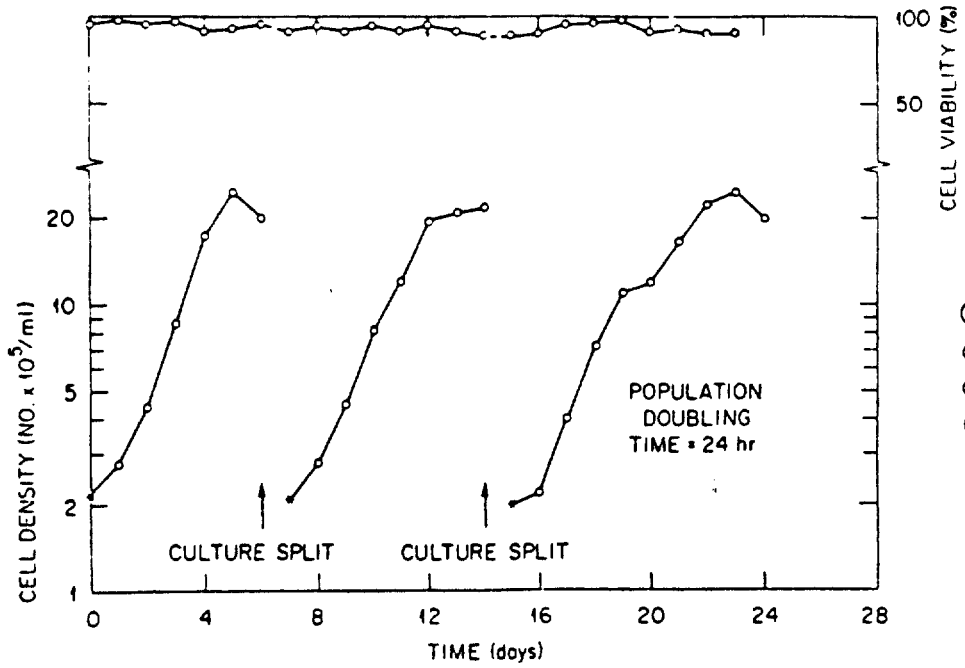
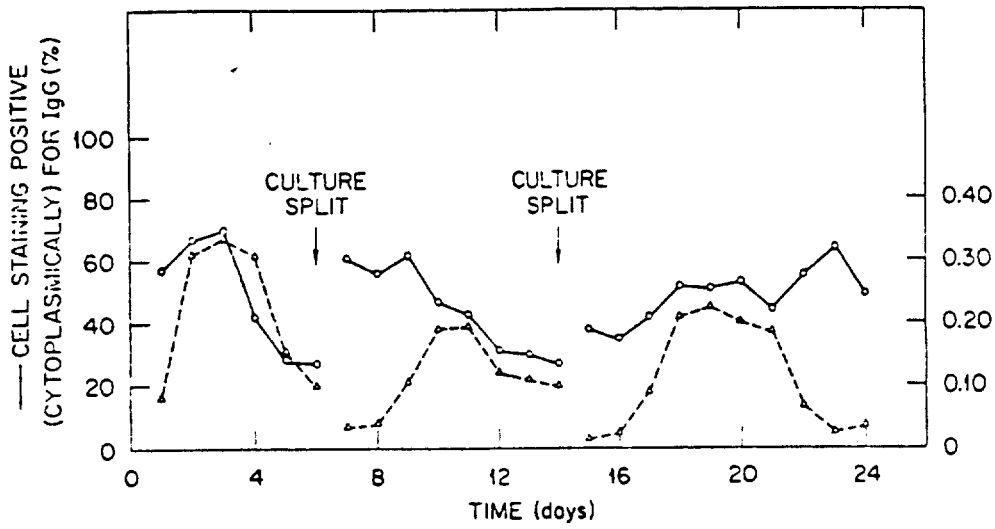


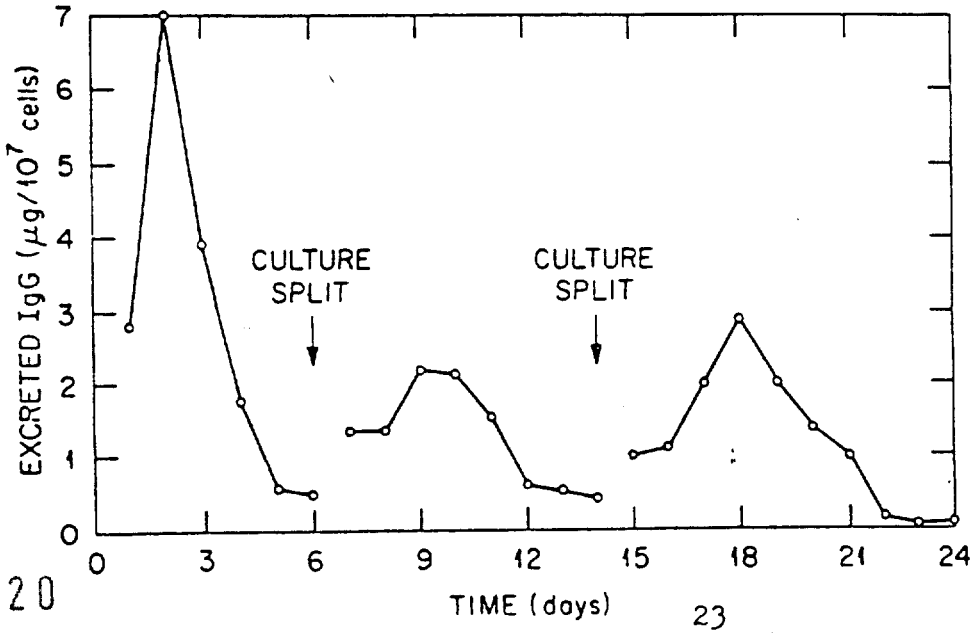
Graph 1

Growth of BF cells under optimum conditions including daily complete medium change.



Graph 2

Variation of IgG secretion (μg/ml) and percentage of IgG-containing cells in BF cultures, as a function of growth. Cell density/day of culture shown in Graph 1. Secreted IgG assayed by quantitative immunofluorescence. IgG-containing cells assayed by flow cytometry, using fluorescent antibody.



Graph 3

Variation in IgG secretion/cell as a function of growth. Cell density/day of culture shown in Graph 1.

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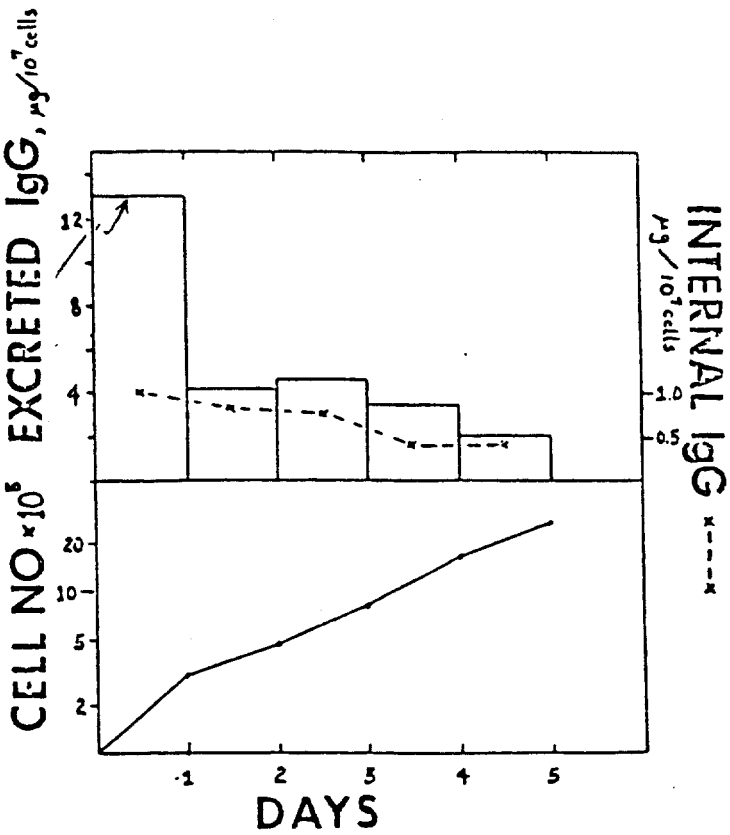
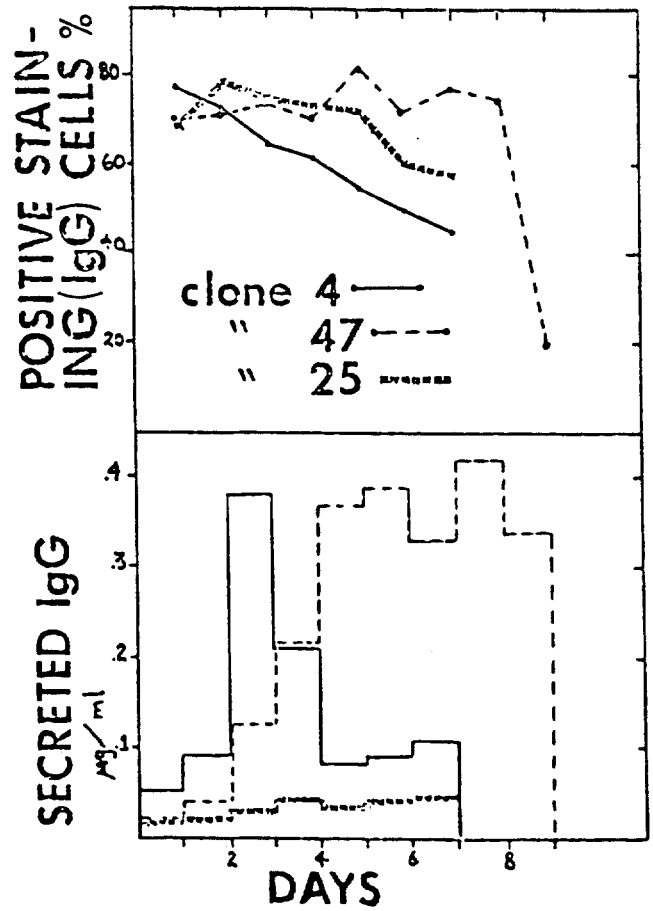


Graph 4

Comparison of secreted and intracellular IgG in BF cells as a function of growth. IgG was assayed by quantitative immunofluorescence. Complete medium changes were made daily.



Graph 5

Comparision of secreted IgG with the percentage of cells containing cytoplasmic IgG in various clones of BF cells. IgG-positive cells were assayed by flow cytometry, and secreted IgG was assayed by quantitative immunofluorescence.

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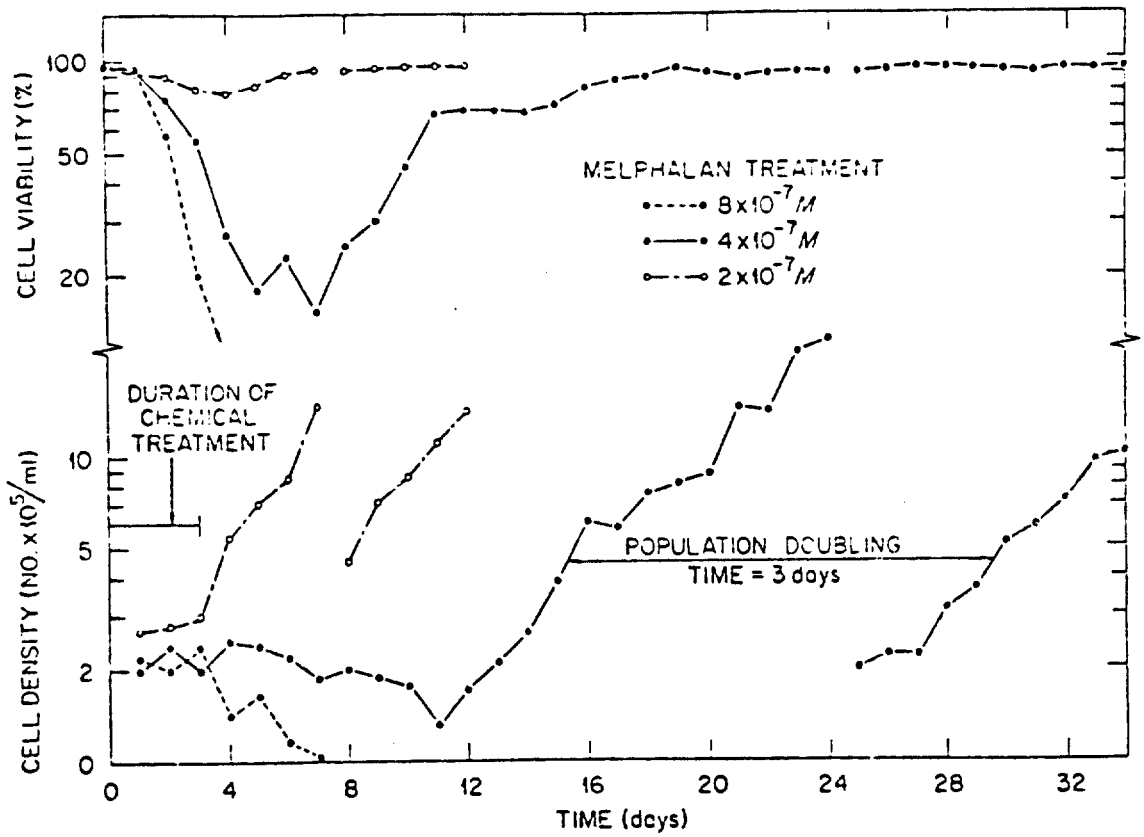
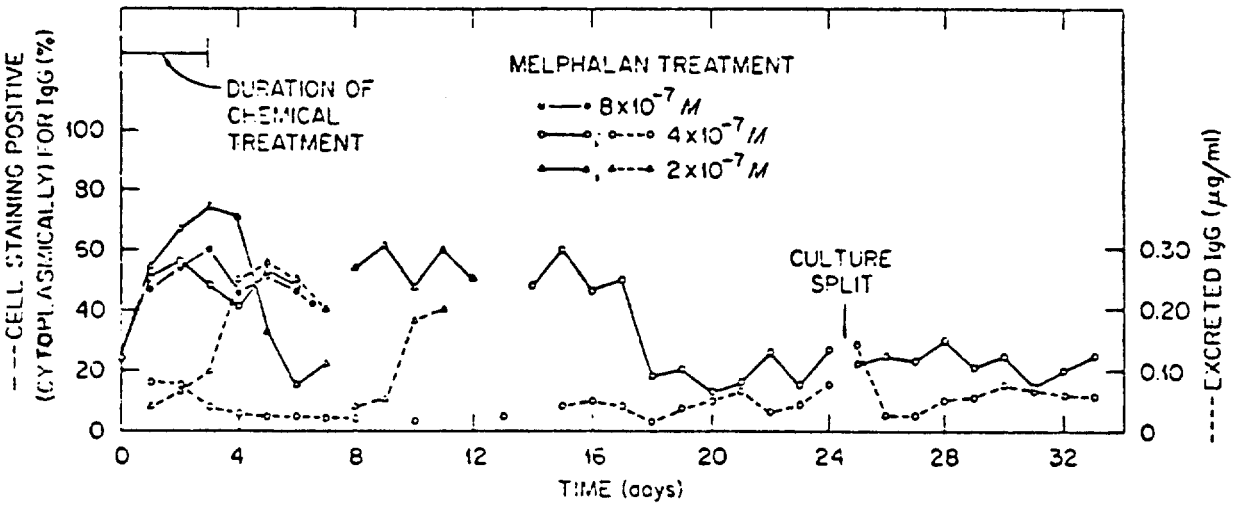


Graph 6

Effect of varying concentrations of Melphalan on viability and growth of BF cells. Cultures treated with 8 x 10^{-7} M Melphalan did not recover. Complete daily medium changes were made on cultures treated with 4 x 10^{-7} M Melphalan following viability recovery.



Graph 7

Alteration of IgG secretion and percentage of cells containing cytoplasmic IgG after Melphalan treatment. Cell density/day of culture shown in Graph 6.

D. Methods

Assays of IgG Synthesis, Secretion and Structure.

(1) Secreted IgG. Medium from cultures of BF cells is collected, dialyzed against distilled water, lyophilized, and reconstituted with water to 1/5 - 1/10 the original volume. The amount of IgG in a suitable aliquot of this solution is determined by using a solid phase immunofluorescence assay specific for human IgG (Immunofluor IgG--Biorad Laboratories). In our laboratory, this assay has been found to detect accurately as little as 50 ng of IgG and to be linear over a range of 50-800 ng of IgG. Fetal calf serum, even in large excess, does not interfere in the assay, nor do free kappa light chains.

(2) Internal (Cytoplasmic) IgG. BF cells are collected and fixed for 20 min. @ $0^{o}C$ in equal volumes of 10% glycerol in phosphate buffered saline and acetone (15). After fixation, cells can be stored for 2-3 wks @ $4^{o}C$ before analysis without significant deterioration, if necessary. An appropriate aliquot (no less than 5×10^{5} or more than 1×10^{6}) of cells is stained with a 1:20 dilution of FITC-conjugated anti-human IgG rabbit antibody (Hyland Diagnostics, Inc.) for 45 min. @ $37^{o}C$ and analyzed by cytofluorometry. The above conditions have been established as optimal for maximal fluorescent staining of the cells. The Hyland antiserum weakly cross-reacts with fetal calf serum and is routinely absorbed with 0.1 volume of fetal calf serum before use. Cytofluorometric analysis of the percentage of fluorescent cells in the total cellular population, using a Biophysics Model 4800A Cytofluorograph with a 10 mW Argon laser, has proven to be a major imporvement over fluorescence microscopy, in terms of quantitation and assay time. Appropriate electronic windows can be established in the cytofluorograph, so that only those cells are counted as positive which produce an electronic signal (proportional to their fluorescence intensity) greater than the signal from fixed cells which are unstained, and which show a small degree of fluorescence from various normal cell constituents. Using this technique, it is possible to count triplicate samples of 1000 cells each in less than 30 seconds. The instrument automatically displays the percentage of cells which fluoresce above the background threshold. Approximate correlations between cytofluorometry and fluorescence microscopy have been found in our laboratory, but lack of any real objectivity in microscopy makes quantitative comparisons difficult.

It should be possible, using a newly acquired argon-ion laser, which produces approximately 1.0 watt of optical power at 488 nm, to obtain accurate fluorescence intensity distributions (by pulse height analysis) for any given population of stained cells, and to relate this distribution to cell size, or other cellular parameters. We then will be able to divide a population of cells into discrete subpopulations of differing fluorescence intensities. The availability to us of a cell-sorting facility (currently being constructed in the Biology Division of ORNL), will permit isolation of subpopulations of differing intensities and such subpopulations can be directly studied for their capacity to synthesize IgG. Alteration of such subpopulations by environmental agents can be investigated.

Using antisera specific for "free" human kappa chains (kindly provided by Dr. Alan Solomon), it will be possible to determine the percentage of BF cells producing only the κ light chain. Alteration of percentage during environmental perturbation can thus be monitored. It will not be possible with our current cytofluorograph to detect, on the single cell level, cells producing both complete IgG and free κ light chains, but the flow cytometry/cell sorting facility of Dr. Paul Mullaney (Biology Division) will make such measurements feasible.

Finally, direct comparisons of percentage of cytoplasmically stained cells and varying levels of associated intensity can be made with actual quantities of IgG con-

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tained in a given population of cells. Cells will be collected, washed thoroughly and lyzed by osmotic shock and sonication. Appropriate aliquots will be assayed for IgG, using the Immunofluor assay.

(3) Surface (Membrane) IgG. BF cells are collected, washed in PBS, and stained with Hyland FITC-conjugated anti-IgG antisera (1:10 dilution in PBS) for 45 min. @ 4°C. Following washing in PBS containing 0.02% sodium azide to prevent capping, the percentage of cells with surface IgG can be assayed by cytofluorometry. Previous experiments to assay surface IgG by the above procedure (using the small 10 mW laser) have indicated a maximum of 12-15% of the cells fluoresce above background levels, although the more usual number is 2-4%. Many of these positive cells are not well-separated in electronic signal from the background threshold. The use of the higher power argon-ion laser should obviate this difficulty. The percentage of cells with membrane-bound κ light chains can also be detected by cytofluorometry, using reagents and procedures previously detailed.

(4) Immunological Probe of IgG Structure. Our anti-idiotypic antisera specific for V region determinants of the BF IgG protein, and commercial antisera (Hyland) specific for the C region of human IgG will be used in standard immune precipitin assays to determine whether the IgG produced by BF cells exposed to environmental agents reacts similarly to IgG from control cells. Since changes in BF IgG structure could occur and not affect its immunoreactivity, we will also compare by isoelectric focusing in polyacrylamide gels IgG isolated from perturbed and control cultures.

Protocol for Immunotoxicity Assay

BF cells will be grown in RPMI 1640, supplemented with 10% fetal calf serum and antibiotics, and at the time of testing will be divided into 2 identical cultures. One of the cultures will be exposed to various concentrations of the environmental agent of interest, while the other culture (lacking the agent) will be treated in the same manner (i.e., with solvent, microsomal enzymes, etc.). Following recovery from any toxic effect (monitored by daily cell count and cell viability, both assayed by cytofluorometry), IgG production from perturbed and control BF cells will be compared by methods detailed above. Cells will be passaged through at least 2 growth cycles for these comparisons.

Environmental agents to be tested first include direct-acting insults such as UV light, ionizing radiation, and directly-acting (those not requiring metabolism to exert toxic effects) chemicals. If the BF cells lack significant cytochrome P_{450} enzyme activity, it will be necessary to use a cell-mediated or microsomal homogenate-mediated assay to activate many chemicals of environmental concern. In the case of BF cells, this can be rather easily accomplished, since they do not attach to plastic. Rat liver cells (provided to our laboratory by I. B. Weinstein) or hamster embryo fibroblasts will be grown on plastic plates, and BF cells plus the chemical of interest can then be added. Following exposure, BF cells can be removed from the attached metabolizing cell layer by washing and trituration. If it seems desirable to use a totally human system, so that any unique metabolites produced by human enzymes can be assayed, human cells such as skin fibroblasts could be used as the attached metabolizing layer. Alternatively, microsomal homogenates from human cells or cell lines known to contain metabolizing enzyme activities could be added, along with chemicals of interest, to BF cell cultures.

When perturbations of IgG production in response to an environmental agent are seen, it will be important to identify the nature of the lesion responsible for this change. For instance, if IgG secretion is diminished, is this due to decreased synthesis, or decreased rate of secretion, or both factors? Radiolabelling of BF IgG by using radioactive amino acids in culture medium lacking these specific amino acids will

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permit comparative studies of IgG synthesis in control and perturbed cells. These studies will be facilitated by the fact that IgG can be rather simply isolated from other cellular proteins by affinity chromatography, using an anti-IgG affinity column.

Future directions of investigation would involve studies directed at determining the subcellular sites at which immunotoxicants exert their deleterious effects.

Safety Precautions

All cell culture work will be carried out in BioHazard hoods. Lasers in the cytofluorograph are totally enclosed, and circuit-breaking interlocks are on the instrument case. Weighing, handling and disposal of carcinogenic or other hazardous chemicals will be carried out according to safety procedures and protocols established by ORNL and the Biology Division.

[redacted]

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IMMUNOGLOBULIN (IgG) PRODUCTION AS A MARKER FOR MUTAGENESIS: COMPARISON OF CHEMICAL ACTIVITY IN LYMPHOID CELLS OF HUMAN AND MOUSE ORIGIN

G.D. Griffin, B.A. Owen, G.D. Novelli,
A. Solomon, and P.J. Walsh
Health and Safety Research and Biology Divisions
Oak Ridge National Laboratory
Oak Ridge, TN

The pattern of synthesis and excretion of IgG by a human lymphoblastoid cell line (BF) has been investigated to determine the action of mutagenic agents in altering IgG structure, synthesis, or secretion. IgG synthesis was detected by cytoplasmic staining with fluorescein-conjugated anti-IgG antisera, and excretion of IgG into culture medium was determined by fluoremetry using Immunofluor Beads (Bio Rad). Both IgG synthesis and excretion are altered in a reproducible fashion by the growth condition of the lymphoblastoid cells. Maximal synthesis and excretion occur with exponential growth. However, when the cells reach a growth plateau phase (no net increase in cell number), IgG synthesis and excretion decline dramatically. Treatment with melphalan, a cancer chemotherapeutic agent and putative mutagen, results in extensive cell death. Following removal of the drug, the remaining viable cells, if allowed to grow, demonstrate apparently permanent alterations in IgG metabolism including (1) a reduction in the excretion of IgG to 1/8-1/4 the levels in untreated cultures, and (2) a decrease in the percentage of cytoplasmically IgG-positive (i.e., IgG-synthesizing) cells to approximately 1/2 the level in control cultures. In this system, we have not detected a change in IgG structure, as assyed for by various immunologically-specific antisera.
(Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract W-7504-eng-26 with the Union Carbide Corporation.)

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IMMUNOGLOBULIN (IgG) PRODUCTION IN A HUMAN LYMPHOBLASTOID CELL LINE: ALTERATION BY MELPHALAN, A CANCER CHEMOTHERAPY AGENT. G. D. Griffin, B. A. Owen, C. Atchley, A. Solomon, P. J. Walsh, and G. D. Novelli. Health and Safety Research and Biology Divisions, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

The possibility of using IgG synthesis and excretion by a human lymphoblastoid cell line (BF) as an *in vitro* indicator of chemical mutagenesis has been studied. Cells positive for cytoplasmic IgG (cytoplasmic$^+$) were measured by flow cytometry using a fluorescein-labeled antibody to IgG, while excreted IgG was measured by an immunofluorescence assay. The percentage of cytoplasmic$^+$ cells and amount of IgG excreted per ml of culture medium increase in exponentially growing cells to maximal values of 70% and 2-3 μg of IgG/ml, respectively. At plateau phase, the percentage of cytoplasmic$^+$ cells and amount of excreted IgG both decline to nearly zero, and this cycle repeats as cells enter another growth phase. When cells were exposed to melphalan at concentrations of 2, 20 or 40 x 10^{-8} M for 72 hrs followed by drug removal, there was a concentration-dependent decrease in cell viability. Cells exposed to the highest concentration of melphalan showed the greatest effects including: (1) decrease in cell viability to 30%, followed by gradual recovery; (2) gradual decrease in percentage of cytoplasmic$^+$ cells to consistent levels of 30%; (3) immediate and persistent decrease in amount of excreted IgG to 1/8-1/4 levels in untreated cultures; and (4) increase in doubling time from 22 hrs to 40 hrs. Cells treated at intermediate concentrations of melphalan showed concentration-dependent effects on IgG excretion, but not consistent changes in percentage of cytoplasmic$^+$ cells.

Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation and USPHS Research Contract CA10056.

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CONTROL OF SYNTHESIS AND EXCRETION OF IMMUNOGLOBULIN (IgG) BY A HUMAN LYMPHOBLASTOID CELL LINE. G. Griffin,* B. Owen,* A. Lynch,* C. Atchley,* P. Walsh,* A. Solomon and G. D. Novelli. Health & Safety Research and Biology Divisions, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

Extensive variability of IgG excretion by a human lymphoblast cell line (BF) prompted studies of IgG control mechanisms. Cell cultures at low density ($5x10^4$/ml) excrete ~ 0.3 μg/ml of IgG, and 40-50% of the cells stain positively for cytoplasmic IgG (IgG^+) with FITC-conjugated anti-human IgG. The percentage of IgG^+ cells and amount of secreted Ig increase to 80-90% and 1.5-2.0 μg/ml, respectively, in exponential growth phase ($5x10^5$/ml). As growth plateau ($2x10^6$/ml) is approached, IgG excretion and IgG^+ cells decrease to 0.3-0.5 μg/ml and 15-30%. This decrease in IgG synthesis (% of IgG^+ cells) and excretion is apparently not due to: (1) depletion of medium component(s); (2) differing IgG synthesis due to cell heterogeneity, since cell clones exhibit the same pattern as the parent cells; (3) differences in IgG synthesis related to cell cycling in plateau vs exponential growth, since cell cycle analysis indicated only small decreases in percentage of cells passing through S and G_2 phases in growth plateau. Available evidence suggests excreted IgG acts as a regulator of IgG synthesis in BF cells.

(Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.)

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1079029

Griffin, G. [redacted]

Attached please find a copy of Dr. Alan Solomon's Consultant Contract with the Health and Safety Research Division. Dr. Solomon will provide consulting services for this grant application as per this Consultant Contract.

1079030

UNION CARBIDE CORPORATION
NUCLEAR DIVISION

April 1, 1981

Dr. Alan Solomon
2705 Riverside Drive
Knoxville, Tennessee 37914

Dear Sir:

Subcontract No. 7080
Supplemental Agreement No. 5

Referring to the subcontract dated March 24, 1976 covering your employment to render advisory and consultant services to Union Carbide Corporation, Nuclear Division, we hereby confirm our understanding that the said subcontract shall be modified as follows, effective April 1, 1981.

The period of performance shall be extended for an additional period beginning April 1, 1981 and ending March 31, 1982.

In Article III - Consideration, paragraph A., subdivision (1) the consideration for consultant services is hereby increased from ONE HUNDRED SEVENTY FIVE DOLLARS ($175.00) per day to ONE HUNDRED NINETY ONE DOLLARS ($191.00) per day.

Delete Articles V-Drawings, Sketches, Memoranda, Etc., VI-Copyright, and VII-Patents in their entirety.

Delete Article XIV - Disputes and substitute the attached new article therefore.

The provisions of the attached Exhibits 1A-Patent Rights (Short Form) (5-80) and 20-Rights In Techincal Data (Short Form) (5-80) are hereby made a part of this subcontract

All other provisions, terms and conditions of the said subcontract shall remain unchanged.

1079031

Please confirm your acceptance of this Supplemental Agreement by signing as indicated below and returning two copies of this Supplemental Agreement to us.

ACCEPTED:

by: _____
Consultant

Very truly yours,

UNION CARBIDE CORPORATION
NUCLEAR DIVISION

BY: _____
Contract Buyer

1079032

As used herein, the word "Seller" shall be deemed to mean "Consultant"

(a) By reason of the Company's entering into this contract as agent for the Government, the Seller is considered a "Contractor" within the meaning of Section 2(4) of the Contract Disputes Act of 1978 (P.L. 95-563) and this contract is subject to such Act.

(b) Except as provided in the Act, all disputes arising under or relating to this contract shall be resolved in accordance with this article.

(c) (1) As used herein, "claim" means a written demand or assertion by one of the parties seeking, as a legal right, the payment of money, adjustment or interpretation of contract terms, or other relief, arising under or relating to this contract.

(2) A voucher, invoice, or request for payment that is not in dispute when submitted is not a claim for the purposes of the Act. However, where such submission is subsequently not acted upon in a reasonable time, or disputed either as to liability or amount, it may be converted to a claim pursuant to the Act.

(3) A claim by the Seller shall be made in writing and submitted to the Contracting Officer of the Company's Contract No. W-7405-eng-26 with the Government for decision. A claim by the Company against the Seller shall be subject to a decision by the Contracting Officer of the Company's Contract No. W-7405-eng-26 with the Government.

(d) For Seller claims of more than $50,000, the Seller shall submit with the claim a certification that the claim is made in good faith; the supporting data are accurate and complete to the best of the Seller's knowledge and belief; and the amount requested accurately reflects the contract adjustment for which the Seller believes the Government is liable. The certification shall be executed by the Seller if an individual. When the Seller is not an individual, the certification shall be executed by a senior company official in charge at the Seller's plant or location involved, or by an officer or general partner of the Seller having overall responsibility for the conduct of the Seller's affairs.

(e) For Seller claims of $50,000 or less, the Contracting Officer must render a decision within 60 days. For Seller claims in excess of $50,000, the Contracting Officer must decide the claim within 60 days or notify the Seller of the date when the decision will be made.

(f) The Contracting Officer's decision shall be final unless the Seller appeals or files a suit as provided in the Act.

(g) The authority of the Contracting Officer under the Act does not extend to claims or disputes which by statute or regulation other agencies are expressly authorized to decide.

(h) Interest on the amount found due on a Seller claim shall be paid from the date the claim is received by the Contracting Officer until the date of payment.

(i) Except as the parties may otherwise agree, pending final resolution of a claim by the Seller arising under the contract, the Seller shall proceed diligently with the performance of the contract in accordance with the Contracting Officer's decision.

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EXHIBIT 1A - PATENT RIGHTS (SHORT FORM) (5-80)

(a) Definitions. (1) "Subject Invention" means any invention or discovery of the Seller conceived or first actually reduced to practice in the course of or under this subcontract, and includes any art, method, process, machine, manufacture, design, or composition of matter, or any new and useful improvement thereof, or any variety of plants, whether patented or unpatented, under the Patent Laws of the United States or any foreign country.

(2) "Patent Counsel" means the DOE Patent Counsel assisting the procuring activity.

(b) Invention disclosures and reports. (1) The Seller shall furnish the Company: (i) A written report containing full and complete technical information concerning each subject invention within 6 months after conception or first actual reduction to practice but in any event prior to any sale, public use, or public disclosure of such invention known to the Seller. The report shall identify this subcontract and inventor and shall be sufficiently complete in technical detail and appropriately illustrated by sketch or diagram to convey to one skilled in the art to which the invention pertains a clear understanding of the nature, purpose, operation, and to the extent known, the physical, chemical, biological, or electrical characteristics of the invention;

(ii) Upon request, but not more than annually, interim reports on a DOE-approved form listing subject inventions for that period and certifying that all subject inventions have been disclosed or that there were no such inventions; and

(iii) A final report on a DOE-approved form within 3 months after completion of the subcontract work listing all subject inventions and certifying that all subject inventions have been disclosed or that there were no such inventions.

(2) The Seller agrees that the Government may duplicate and disclose subject invention disclosures and all other reports and papers furnished or required to be furnished pursuant to this subcontract.

(c) Allocation of principal rights. (1) Assignment to the Government. The Seller agrees to assign to the Government the entire right, title, and interest throughout the world in and to each subject invention, except to the extent that rights are retained by the Seller under paragraphs (c)(2) and (d) of this clause.

(2) Greater rights determinations. The Seller, or the employee-inventor with authorization of the Seller, may request greater rights than the nonexclusive license and the foreign patent rights provided in paragraph (d) of this clause on identified inventions in accordance with the procedure and criteria of 41 CFR 9-9.109-6. A request for a determination whether the Seller or the employee-inventor is entitled to retain such greater rights must be submitted to the Company (with notification by the Company to the Patent Counsel) at the time of the first disclosure of the invention pursuant to paragraph (b)(1) of this clause or not later than 9 months after conception or first actual reduction to practice, whichever occurs first, or such longer period as may be authorized by the Company (with notification by the Company to the Patent Counsel) for good cause shown in writing by the Seller. The information to be submitted for a greater rights determination is specified in 41 CFR 9-9.109-6(e).

(d) Minimum rights to the Seller. The Seller reserves a revocable, non-exclusive, paid-up license in each patent application filed in any country on a subject invention and any resulting patent in which the Government acquires title. Revocation shall be in accordance with the procedures of paragraphs (c)(2) and (3) of the clause in 41 CFR 9-9.107-5(a). The Seller also has the right to request foreign rights in accordance with the procedures of paragraph (c)(4) of the clause in 41 CFR 9-9.107-5(a).

(e) Employee and subcontractor agreements. Unless otherwise authorized in writing by the Company, the Seller shall: (1) Obtain patent agreements to effectuate the provisions of the Patent Rights clause from all persons who perform any part of the work under this subcontract except nontechnical personnel, such as clerical employees and manual laborers.

(2) Unless otherwise authorized or directed by the Company, the Seller shall include the Patent Rights clause of 41 CFR 9-9.107-5(a) or 41 CFR 9-9.107-6, as appropriate, modified to identify the parties in any subcontract hereunder having as a purpose the conduct of research, development or demonstration work; and

(3) Promptly notify the Company in writing upon the award of any subcontract containing a Patent Rights clause by identifying the subcontractor, the work to be performed under the subcontract, and the dates of award and estimated completion. Upon the request of the Company the Seller shall furnish a copy of the subcontract to the Company.

(f) Atomic energy. (1) No claim for pecuniary award or compensation under the provisions of the Atomic Energy Act of 1954, as amended, shall be asserted by the Seller or its employees with respect to any invention or discovery made or conceived in the course of or under this subcontract.

(2) Except as otherwise authorized in writing by the Company, the Seller will obtain patent agreements to effectuate the provisions of paragraph (f)(1) of the clause from all persons who perform any part of the work under this subcontract except nontechnical personnel such as clerical employees and manual laborers.

(g) Publication. In order that information concerning scientific or technical developments conceived or first actually reduced to practice in the course of or under this subcontract is not prematurely published so as to adversely affect patent interests of DOE, the Seller agrees to submit to the Company for patent review a copy of each paper 60 days prior to its intended publication date. The Seller may publish such information after expiration of a 60-day period following such submission or prior thereto if specifically approved by the Company, unless the Seller is informed that in order to protect patentable subject matter, publication must be further delayed.

EXHIBIT 20 - RIGHTS IN TECHNICAL DATA - SHORT FORM (5-80)

(a) Definitions. The definitions of terms set forth in 41 CFR 9-9.201 apply to the extent these terms are used herein.

(b) Allocations of rights. (1) The Government shall have: (i) Unlimited rights in technical data first produced or specifically used in the performance of this subcontract;

(ii) The right of DOE or its representatives to inspect at all reasonable times up to three years after final payment under this subcontract all technical data first produced or specifically used in this subcontract (for which inspection the Seller or its subcontractor shall afford proper facilities);

(iii) The right to have any technical data first produced or specifically used in the performance of this subcontract delivered to the Government as the Company may from time to time direct during the progress of the work or in any event as the Company shall direct upon completion or termination of this subcontract.

(2) The Seller shall have the right to use for its private purposes, subject to patent, security or other provisions of this subcontract, technical data it first produces in the performance of this subcontract provided the data requirements of this subcontract have been met as of the date of the private use of such data. The Seller agrees that to the extent it receives or is given access to proprietary data or other technical, business or financial data in the form of recorded information from DOE or a DOE contractor or subcontractor, the Seller shall treat such data in accordance with any restrictive legend contained thereon, unless use is specifically authorized by prior written approval of the Company.

(c) Copyrighted material. (1) The Seller agrees to, and does hereby grant to the Government, and to its officers, agents, servants and employees acting within the scope of their duties:

(i) A royalty-free, nonexclusive, irrevocable license to reproduce, translate, publish, use, and dispose of and to authorize others so to do, all copyrightable material first produced or composed in the performance of this subcontract by the Seller, its employees, or any individual or concern specifically employed or assigned to originate and prepare such material; and

(ii) A license as aforesaid under any and all copyrighted or copyrightable works not first produced or composed by the Seller in the performance of this subcontract but which are incorporated in the material furnished under this subcontract, provided that such license shall be only to the extent the Seller now has, or prior to completion or final settlement of this subcontract may acquire, the right to grant such license without becoming liable to pay compensation to others solely because of such grant.

(2) The Seller agrees that it will not knowingly include any material copyrighted by others in any written or copyrightable material furnished or delivered under this subcontract without a license as provided for in subparagraph (1)(ii) hereof, or without the consent of the copyright owner, unless it obtains specific written approval of the Company for the inclusion of such copyrighted material.

RETURN ALL THREE CARDS WITH APPLICATION

This card will be retained in the Division of Research Grants

This card will be returned to the addressee as soon as the Initial Review Group assignment is made.

The summary statement pertaining to the review of the application will be sent to the addressee approximately 2 weeks after the National Advisory Council/Board meets. See application instructions for the review and award schedule.

This card will be returned to the addressee to acknowledge receipt of the application.

TO BE COMPLETED BY APPLICANT

NAME OF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR OR AWARD CANDIDATE *(Last, First, Initial)*

Griffin, Guy D.

SPONSORING APPLICANT ORGANIZATION *(Name and address)*

Department of Energy
Oak Ridge Operations
Post Office Box E
Oak Ridge, TN 37830

$ 66,700

AMOUNT REQUESTED *(1st yr.) (Omit for Fellowships)*

DEPARTMENT *(if applicable)*

Health and Safety Research Division
Health Effects and Epidemiology Group

AGENCY USE ONLY

DATE RECEIVED

TITLE OF APPLICATION

Human Cell Assay of Environmental Immunotoxicants

PHS-3830
Rev. 5-80

APPLICATION RECEIPT RECORD

INSTRUCTIONS TO APPLICANT: Complete the return address of the Principal Investigator/Program Director or Award Candidate on the reverse side. Title of application must appear below.

TITLE Human Cell Assay of Environmental Immunotoxicants

Your application has been assigned for initial scientific merit review to the group indicated below:

NOTIFY YOUR BUSINESS OFFICE OF THE FOLLOWING: ASSIGNMENT NO: _____

NOTE: For Individual National Research Service Awards, Research Career Development Awards, and New Investigator Research Awards, make sure that reference letters are sent in immediately after you are notified about the Initial Review Group assignment.

PHS-3830
Rev. 5-80

TO BE COMPLETED BY APPLICANT

(Also complete address on reverse side)

TITLE OF APPLICATION

Human Cell Assay of Environmental Immunotoxicants

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR OR AWARD CANDIDATE

Guy David Griffin

DATE RECEIVED

Your application was received on the date indicated.

1079037

PHS-3830
Rev. 5-80

APPLICATION RECEIPT RECORD

DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Dr. Guy D. Griffin
Health and Safety Research Division
Oak Ridge National Laboratory
P. O. Box X, Building 4500S
Oak Ridge, TN 37830

PHS-3830 (Back)
Rev. 5-80

DEPARTMENT OF
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Dr. Guy D. Griffin
Health and Safety Research Division
Oak Ridge National Laboratory
P. O. Box X, Building 4500S
Oak Ridge, TN 37830

PHS-3830 (Back)
Rev. 5-80

1079038

CHECKLIST

This is the required last page of the application.

Check the appropriate boxes and provide the information requested.

TYPE OF APPLICATION:

[x] NEW application *(This application is being submitted to the PHS for the first time.)*

[ ] COMPETING CONTINUATION of grant number: _____.
(This application is to extend a grant beyond its original project period.)

[ ] SUPPLEMENT to grant number: _____.
(This application is for additional funds during a funded project period.)

[ ] REVISION of application number: _____.
(This application replaces a prior version of a new, competing continuation or supplemental application.)

[ ] Change of Principal Investigator/Program Director.
Name of former Principal Investigator/Program Director: _____.

ASSURANCES IN CONNECTION WITH:

Civil Rights	Handicapped Individuals	Sex Discrimination	Human Subjects General Assurance *(If applicable)*	Laboratory Animals *(If applicable)*
[x] Filed	[x] Filed	[X] Filed	[ ] Filed	[ ] Filed
[ ] Not filed	[ ] Not filed	[ ] Not filed	[ ] Not filed	[ ] Not filed

INDIRECT COSTS:

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office. If the applicant organization is in the process of initially developing or renegotiating a rate, or has established a rate with another Federal agency, it should, immediately upon notification that an award will be made, develop a tentative indirect cost rate proposal based on its most recently completed fiscal year in accordance with the principles set forth in the pertinent *DHHS Guide for Establishing Indirect Cost Rates*, and submit it to the appropriate DHHS Regional Office. Indirect costs will not be paid on foreign grants, construction grants, and grants to individuals, and usually not on grants in support of conferences.

[x] DHHS Agreement Dated: Feb. 14, 1979
_____ % Salary and Wages or 35 % Total Direct Costs.

Is this an off-site or other special rate, or is more than one rate involved? [ ] YES [x] NO
Explanation: _____

[ ] DHHS Agreement being negotiated with _____ Regional Office.

[ ] No DHHS Agreement, but rate established with _____ Date_____

[ ] No Indirect Costs Requested.