ANNUAL REPORT OF CLINICAL RESEARCH ACTIVITIES

October 1, 2000 to September 30, 2001

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health
Warren Grant Magnuson Clinical Center

For Administrative Use

DIRECTOR'S MESSAGE

Whereas NIH Clinical Center staff provide an impressive range of research support and services to institute clinical researchers, investigators from the Clinical Center also conduct independent clinical research activities. Last year, Clinical Center researchers received wide recognition through publications of these research efforts. As an example, an article in the *Journal of the American Medical Association* by Critical Care Medicine Department Fellow, Dr. Luciana Borio, assessing the two fatal cases of inhalation anthrax that occurred in District of Columbia postal workers, received significant media attention. In addition, Dr. Brad Wood's research on tumor ablation therapy was highlighted by the Radiological Society of North America for media release.

This report summarizes clinical research projects overseen by Clinical Center investigators in FY 2001.

John I. Gallin, M.D.
Director, Warren Grant Magnuson Clinical Center
National Institutes of Health

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ANESTHESIA AND SURGICAL SERVICES DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL008046-01

LBC: ANES

Title: The Role of Autonomic Innervation: The Interactions of Opioids and β-Agonists

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Hugh L. Preas, II, M.D.

Supervisor of Record: Henry Masur, M.D.

Collaborators, Lab: Charles Natanson, M.D. (CCM, CC)
Stephen Richmond (CCM, CC)
Steven Solomon, Ph.D. (CCM, CC)

Total Staff Years: .15

Human Research: Neither human cells nor tissues

Keywords: Fentanyl, Morphine, Vagotomy, Isoproterenol, Halothane

Summary: Fentanyl is a commonly used narcotic that has wide applications in general anesthesia and critical care settings. We have previously shown that fentanyl, an opioid agonist, when administered to canines: (1) blocked β-adrenergic hemodynamic responses to epinephrine and isoproterenol; and (2) did not affect the α-adrenergic effects of epinephrine or phenylephrine. These studies were extended to define the mechanism of fentanyl-catecholamine interactions by performing similar studies in canines with a vagotomy. These studies have implications for understanding the mechanism that accounts for altered hemodynamic responses due to fentanyl in anesthesia and in critically ill patients.

CLINICAL BIOETHICS DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL010501-03

LBC: CB

Title: Survey of Organ Procurement Organizations

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Lab: Neal Dickert, B.A. (CBD, CC)
David S. Wendler, Ph.D. (CBD, CC)

Total Staff Years: 1

Human Research: Human subject research: Interviews

Keywords: Organ Procurement Organizations

Summary: This protocol is designed to determine organ procurement organizations' practices and policies regarding consent (family or individual) for cadaveric organ donation and the reasons behind these practices and policies. The study has been approved by the Institute Review Board. Since we are proposing to survey more than nine individuals with whom we do not have an existing clinical relationship, this project falls under the Paperwork Reduction Act and thus had to receive Office of Management and Budget approval. Approval was granted. Sixty-one individuals were surveyed. Surveying is completed, and we are currently writing up the results.

LBC: CB

Title: Survey of Individuals At Risk for Alzheimer's Disease

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborator, Lab: David S. Wendler, Ph.D. (CBD, CC)

Total Staff Years: 1

Human Research: Human subject research: Interviews

Keywords: Alzheimer's Disease

Summary: This protocol seeks to survey individuals at risk for Alzheimer's disease with respect to their (1) willingness to participate in clinical research should they develop Alzheimer's disease, (2) willingness to utilize research advance directives, (3) attitudes toward research with stored tissues, (4) attitudes toward confidentiality of research results, and (5) experience with genetic counseling. We surveyed 504 individuals. Surveying is completed, and we are currently writing up the results.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010503-03

LBC: CB

Title: Influences on HIV-infected Subjects' Willingness to Participate in Research and Ability to Give Informed Consent

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Christine P. Grady, Ph.D.

Supervisor of Record: Ezekiel Emanuel, M.D., Ph.D.

Collaborator, Lab: Neal Dickert, B.A. (CBD, CC)

Collaborators, NIH: Grace G. Kelly, R.N. (CC)
Jill A. Lietzau (HAMB, NCI)
Diane M. Rockkress (CC)

Total Staff Years: 1

Human Research: Human subject research: Interviews

Keywords: HIV, Informed Consent

Summary: Protocol was terminated in May 2000. We had some difficulty recruiting an adequate number of subjects from comparable protocols. Nonetheless, we obtained interesting data about the motivations and experience of informed consent from 41 subjects. Data are being analyzed. This study is completed and manuscript is in development.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010504-03

LBC:	CB
Title:	Policies and Practices of Payment to Research Subjects
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Christine P. Grady, Ph.D.
Supervisor of Record:	Ezekiel Emanuel, M.D., Ph.D.
Collaborator, Lab:	Neal Dickert, B.A. (CBD, CC)
Total Staff Years:	1
Human Research:	Neither human cells nor tissues
Keywords:	Payments to Research Subjects, Policies, Practices, Research Subject Payments

Summary: We have completed a review and analysis of policies and guidelines regarding payment of research subjects from 32 research organizations around the country. A manuscript has been submitted for publication. We have also completed a review of 467 randomly selected protocols obtained through the Institute Review Boards of 11 different organizations. Data are being analyzed. The study is completed. One manuscript entitled "Paying Research Subjects: An Analysis of Current Policies" was accepted for publication in the *Annals of Internal Medicine* (predicted publication date, December or January). A second manuscript is still in development.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010505-03

LBC: CB

Title: Patient Perspectives on Health Insurance

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Marion Danis, M.D.

Supervisor of Record: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Extramural:
Richard Duke, Ph.D., Multilogue Corporation
Richard Duke & Associates
Susan Dorr Goold, M.D., M.S.H.A., M.A.
Regents of University Michigan, University of Michigan
Charles Hall, Multilogue Corporation, Richard Duke & Associates
Vana Prewitt Praxis Learning Systems

Total Staff Years: .2

Human Research: Human subject research: Interviews

Keywords: Health Insurance, Patient Perspectives

Summary: This protocol was intended to (1) design a research tool (simulation model) to facilitate group decision making and (2) utilize this tool to examine how patients enrolled in managed care organizations would choose to define their health insurance benefit package. Thus far, the study instrument has been designed and has been pilot tested. The study design has been completed. Study subjects were recruited, and 50 group exercises have been conducted. Data collection was complete on June 23, 2000. Data are being processed and data analysis has been completed. A manuscript is being prepared.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010506-03

LBC:	CB
Title:	Defining a Health Insurance Package for the Uninsured
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Marion Danis, M.D.
Supervisor of Record:	Ezekiel Emanuel, M.D., Ph.D.
Collaborators, Extramural:	Andrea Biddle, Ph.D., M.P.H. Health Policy and Administration, University of North Carolina–Chapel Hill Susan Dorr Goold, M.D., M.S.H.A., M.A. Regents of University Michigan, University of Michigan Vana Prewitt, Praxis Learning Systems
Total Staff Years:	.2
Human Research:	Human subject research: Interviews
Keywords:	Uninsured, Health Insurance Package

Summary: This protocol is designed to determine how uninsured patients would choose to define their health insurance benefit package. Thus far, the study instrument has been designed and has been pilot tested. The study design has been completed. Patient recruitment was scheduled to begin in July 1999. Study subjects were recruited and 20 group exercises have been conducted. Data collection was complete as of June 4, 2000. Data analysis has been completed. Two abstracts have been presented at national meetings. A manuscript has been accepted pending revisions.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010507-03

LBC: CB

Title: Physician Resolution of Ethical Problems

Dates: from 10/01/2000 to 09/30/2001

Lead Investigator: Marion Danis, M.D.

Supervisor of Record: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Extramural:
Brian Mi, Clarridge Center for Survey Research
University of Massachusetts
Gordon DuVal, S.J.D., Center for Addiction and Mental Health
University of Toronto

Total Staff Years: .6

Human Research: Human subject research: Interviews

Keywords: Ethical Problems, Physicians

Summary: This protocol is designed to identify the most frequent and difficult ethical problems encountered by physicians; to examine how physicians resolve these ethical problems; to examine how physicians utilize ethics consultation services; and to determine what barriers or deterrents physicians perceive in utilizing ethics consultation services. Data collection for this study has now been completed. Several analyses have been completed and several are ongoing. One manuscript has been published, one has been submitted, and two are in preparation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010508-03

LBC: CB

Title: Comparison of End-of-Life Costs Between Managed Care and Fee-for-Service

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Extramural: Arlene Ash, Ph.D., Health Care Research Unit, Boston University
Gail Gazelle, M.D., Harvard Pilgrim Health Care
Wei Yu, Ph.D., Health Care Research Unit, Boston University

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Keywords: End-of-Life Costs, Fee-for-Service

Summary: This study is designed (1) to determine the comparative costs and resource utilization over the last year of life for patients treated by managed care, fee-for-service, and Medicare; (2) to compare sites of death, (3) to compare utilization of hospice and hospital beds; and (4) to compare care provided to those over age 65 and those under age 65. A full set of data has been obtained from two managed care companies and from Medicare. Some problems with the data sets are that use of hospice is not fully recorded in one data set and hospitalization prior to death is not fully recorded in another. However, preliminary observations indicate (1) no difference between managed care and fee-for-service in site of death (i.e., hospital versus home) and (2) managed care has a slightly higher proportion of cancer deaths than deaths from heart disease. Full results are expected within the next 12 months. Phase I is completed. We are now entering Phase II, in which we will pursue an interesting finding that African Americans receive more end-of-life care than whites. We will investigate the differences in services provided, the differences in sites of death, and the differences in causes of death, using 2000 data.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010509-03

LBC: CB

Title: Protecting Communities in Biomedical Research

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborator, Extramural: Charles Weijer, M.D., Ph.D., Office of Bioethics Education and Research, Dalhousie University

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Keywords: Biomedical Research

Summary: This project, performed in two phases, examines protections that should be afforded to communities in research. In the first phase, existing protections for communities have been identified and analyzed. This analysis indicates that there are 17 different declarations about protecting communities that delineate five broad groups with a total of 23 different protections. These protections are appropriate to aboriginal communities, such as Native American communities, but do not necessarily seem appropriate to other types of communities. The second phase focuses on determining what protections are appropriate for different types of communities. This Phase entails four steps:(1) delineating the key characteristics that define communities; (2) using these characteristics to develop a typology of different types of communities (this step recognizes that the term “communities” encompasses a diverse set of groups that are not necessarily homogenous—seven different types of communities are identified); (3) delineating the 23 protections identified for the aboriginal communities and indicating the type of characteristics necessary to implement each protection; and (4) linking the different types of communities to appropriate protections through the characteristics that are shared, permitting the definition of the types of protections in research that are appropriate to the different types of communities. The first phase of research has been submitted for publication; the second phase of research is ongoing. This project is completed.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010510-03

LBC: CB

Title: BEST: Best Ethical Strategies in Managed Care

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Lab: Stephen A. Green, M.D. (CBD, CC)
Lauren B. Randel (CBD, CC)

Collaborators, Extramural: Steve Pearson, M.D., M.P.H., Center for Ethics in Health Care
Harvard Pilgrim Health Care
James E. Sabin, M.D., Center for Ethics in Managed Care
Harvard Pilgrim Health Care

Total Staff Years: 4

Human Research: Neither human cells nor tissues

Keywords: Managed Care

Summary: This project begins with the observation that most of the controversy about managed care—such as gag rules, financial incentives, limits on care, and confidentiality—can be viewed as ethics problems. It is unlikely that criticism, a patients' bill of rights, or other approaches will get managed care organizations to be more ethical. The BEST project is based on the idea that showing managed care organizations a list of best practices regarding ethical issues will encourage them to adopt these practices. A consortium of 12 managed care plans was established, including for-profit, not-for-profit, academic, "Blues," and religious-based plans. Nine ethical dimensions were identified: (1) community benefit, (2) care of vulnerable populations, (3) end-of-life care, (4) confidentiality, (5) organization ethics, (6) benefit design and adjudication, (7) technology assessment, (8) financial incentives, and (9) member disclosure and participation. So far, four site visits have been completed, and preliminary best practices have been identified for a number of areas. A conference delineating best practices was held in February 2000. The study is completed. A book contract has been signed with Oxford University Press.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010511-03

LBC: CB

Title: Managing Pharmacy Benefits in Managed Care

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Lab: Lauren B. Randel (CBD, CC)
Karen Titlow, M.A. (CBD, CC)

Total Staff Years: 1

Human Research: Neither human cells nor tissues

Keywords: Managed Care

Summary: One of the major dilemmas facing managed care organizations and health insurers is managing pharmacy benefits. Many new drugs are being introduced, drug prices are rising rapidly, and the pressure to cover pharmacy costs is intense. The dilemma has been most clearly manifested in the controversy surrounding the coverage of Viagra. Data collection has been completed and analyzed. This study examines what coverage decisions insurers make and the information and processes used in making these decisions. Fifty-three organizations—differing in size, tax status, and region—were asked about their policies for four new and controversial drugs:Viagra, Enbrel, Zyban, and Celebrex. Enbrel and Celebrex were much more likely to be covered than Viagra and Zyban. In addition, coverage of Enbrel and Celebrex was limited, through strategies such as prior authorization, to encourage medically appropriate use of these agents, whereas coverage of Viagra and Zyban was limited predominantly through generalized exclusion or through restrictions on quantity or duration of use. Value judgments, rather than cost, seem to play a central, though largely unspoken, role in these coverage decisions. The study is completed.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010512-02

LBC:	CB
Title:	Minnesota CHAT: Public Perspectives on Health Insurance in Minnesota
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Marion Danis
Supervisor of Record:	Ezekiel Emanuel, M.D., Ph.D.
Collaborators, Extramural:	Ellen Benavides, Health Strategies Group Andrea Biddle, Ph.D., M.P.H., Health Policy and Administration University of North Carolina–Chapel Hill Susan Dorr Goold, M.D., M.S.H.A., M.A. Regents of University Michigan, University of Michigan
Total Staff Years:	.2
Human Research:	Human subject research: Interviews
Keywords:	Health Insurance, Minnesota CHAT

Summary: This protocol is designed to determine how residents of Minnesota would choose to define their health insurance benefit package. The results are intended to inform the managed care industry in Minnesota to attend to rising costs and dissatisfaction with choices in health care. The study instrument has been designed. The study design has been completed. Participant recruitment is ongoing. Thirty group exercises were expected to be complete by July 19, 2000. Data processing and data analysis is complete, and a manuscript is being prepared for publication.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010513-02

LBC: CB

Title: A Comparative Study of Ethical Issues in Multinational Clinical Research

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Lab: Christine P. Grady, Ph.D. (CBD, CC)
John Y. Killen (CBD, CC)
David S. Wendler, Ph.D. (CBD, CC)

Total Staff Years: 3

Human Research: Human subject research: Interviews

Keywords: Ethical Issues, Multinational Clinical Research

Summary: This study seeks to inform deliberation and resolution of ethical issues related to multinational clinical research through interviewing various participants of the ESPRIT study. ESPRIT is a multinational collaborative clinical trial of Interleukin-2 in HIV disease. Our study will interview four groups participating in ESPRIT:(1) chairs of the Institute Review Board (IRB) or REC, which reviewed ESPRIT; (2) principal investigators implementing ESPRIT; (3) persons who negotiated the Cooperative Project Assurances with the U.S. government; and (4) selected subjects participating in ESPRIT. The purpose is to compare their attitudes and experiences regarding important ethical issues associated with ESPRIT. Protocol has been reviewed by the NIAID IRB. Survey instruments are being developed and pretested. There has been substantial controversy about the ethics of human subject research in developing countries. This study is designed to provide an ethical framework for clinical research in developing countries and investigate empirically some of the more controversial issues in this research. In conjunction with African clinical researchers, we are examining the question of what constitutes benefits of clinical research and what level of benefits is necessary for ethical multinational research. Similarly, we are trying to define a full set of principles and benchmarks to provide a general framework for determining when clinical research in developing countries is ethical. The empirical research is focusing on whether subjects in African countries are coerced into participating in research; whether they understand the details, including the risks and benefits, of the research; and how participants in clinical research perceive the benefits of the research.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010514-02

LBC: CB

Title: Promises of Benefit in Phase I Oncology Informed Consent Forms

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ezekiel Emanuel, M.D., Ph.D.

Collaborators, Lab: Christine P. Grady, Ph.D. (CBD, CC)
Sam Horng, B.A. (CBD, CC)
Jonathan Rackoff, B.A. (CBD, CC)
Benjamin S. Wilfond, M.D. (CBD, CC)

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Keywords: Informed Consent

Summary: This protocol will analyze consent forms for Phase I oncology trials to assess the manner in which the nature, risks, and potential benefits are communicated. Phase I consent forms are being sought from all of the NCI-designated comprehensive cancer centers and from major pharmaceutical companies that conduct Phase I oncology trials. Institutions have been contacted by phone and are sending consent forms. Data gathering is just beginning. Phase I oncology clinical trials are ethically controversial because they typically involve terminally ill patient-subjects and offer almost no prospect of direct benefit. Studies interviewing Phase I cancer subjects show that many of them expect to benefit from these trials. To evaluate how the description of research purpose and the promise of direct benefit is communicated to subjects, we compiled all 1999 Phase I oncology consent forms from 80 percent of the NCI-designated cancer centers and from six of the top ten cancer pharmaceutical manufacturers. With a scoring instrument, we evaluated five domains in the consent forms:(1) the descriptive properties of the trial, (2) the research purpose and procedures, (3) the promise of benefits, (4) the risks, and (5) the alternatives. We have completed the scoring of the consent forms and are performing a statistical analysis on the data. Preliminary results indicate that no consent forms promised direct benefits to subjects. Many forms (87 percent) qualified the prospect of benefit as uncertain, and almost all forms discuss risk comprehensively, including a mention of death (70 percent). Finally, nearly all forms (90 percent) state that the purpose of the trial is to test safety.

CRITICAL CARE MEDICINE DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL000036-14

LBC: CCM

Title: Studies on the Role of Interleukin-2 in the Management of HIV Infection

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Kovacs, M.D.

Collaborators, Lab: Doreen G. Chaitt (CCM, CC)
Henry Masur, M.D. (CCM, CC)

Collaborators, NIH: Richard Davey, Jr., M.D. (CRS, NIAID)
Judith Falloon, M.D. (CMRS, NIAID)
Henry Lane, M.D. (OCR, NIAID)
Michael Polis, M.D. (CMRS, NIAID)
Jorge L. Tavel, M.D. (DIR, NIAID)
Susan Vogel, R.N. (CMRS, NIAID)

Total Staff Years: 1

Human Research: Human subject research

Keywords: Interleukin-2, HIV Infection

Summary: Interleukin-2 (IL-2) is a cytokine with important regulatory properties or both T and B cells. The current studies were undertaken to evaluate IL-2 in the treatment of HIV infection. Our studies initially focused on patients with CD4 counts above 200 cells/mm3, administering IL-2 for 5 days approximately every 2 months at doses ranging from 6 to 18 million units/d. The courses of IL-2 were well tolerated, although most of the patients required dosage reductions due to IL-2 related adverse effects. Sustained improvement in CD4 number was seen primarily in patients with greater than 200 CD4 cells/mm3. There also was a transient increase in viral load as measured by the bDNA assay seen at day 6 to day 8 following initiation of IL-2 therapy. Responses in CD4 number were less common in patients with lower baseline CD4 counts. Based on the preliminary results seen in our open trial, we undertook a randomized trial to evaluate IL-2 therapy in patients with CD4 counts above 200 cells/mm in combination with currently approved antiretroviral therapies. The study opened in April 1993 and was completed in February of 1995, with 60 patients enrolling. This study also showed in a controlled setting, that intermittent therapy with IL-2 can lead to a substantial and sustained increase in CD4 cell counts without leading to an increase in plasma viral load. More recently, we have focused on improving the tolerance of IL-2, by decreasing the dose and duration of therapy and by evaluating alternative methods of administering IL-2. We had enrolled patients in an extension phase of ongoing studies to determine whether administration of corticosteroids with IL-2 can lead to improved tolerance of IL-2 without interfering with the immunomodulatory effects. This phase has been discontinued due to the occurrence of avascular necrosis of the hip in some patients receiving prednisone. We continue to follow patients receiving IL-2 to determine the long-term side effects and immunologic activity of IL-2. These studies are potentially important because they are the first to suggest that immunomodulating agents combined with antiretroviral agents may have a benefit in patients with HIV infection.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000037-14

LBC: CCM

Title: The Characterization of *Pneumocystis carinii* Surface Antigens

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Kovacs, M.D.

Collaborators, Lab: Lisa Bishop (CCM, CC)
Geetha Kutty (CCM, CC)

Total Staff Years: 1

Human Research: Human cells or tissues

Keywords: *Pneumocystis Carinii*, Surface Antigens

Summary: *Pneumocystis carinii* is a major opportunistic pathogen of immunocompromised patients. Because *P. carinii* cannot be reliably cultured, molecular approaches have been utilized to identify and characterize antigens of this organism. Recombinant antigens can then be used to examine host immune responses to *P. carinii* infections. We have an ongoing project to characterize the antigens of both rat and human *P. carinii*. We have previously purified the major surface glycoprotein (MSG) of both rat and human pneumocystis using HPLC. It is necessary to use *P. carinii* from both sources, because antigenically they are different and specifically the major surface antigen in rat and human *P. carinii* are clearly different. Subsequently, we identified a number of clones from a cDNA library of rat *P. carinii* that contain genes encoding for the MSG. These clones are clearly related but not identical, demonstrating that multiple genes encode the MSG. We have continued studies to characterize potential antigens of *P. carinii* genes. We have cloned a number of human *P. carinii* MSG genes and have expressed a full-length MSG in two fragments. Over the past year we have developed an ELISA to examine antibody responses to these antigens. Using the ELISA, we examined sera from patients with or without HIV infection, and with or without a history of PCP, as well as from a variety of healthy controls. We will continue these studies to better understand the epidemiology of *P. carinii* infection in humans. We have also identified the unique expression site of MSG in human *P. carinii* and can now identify the MSG variants that are expressed in a patient with PCP. The goal of this study is to better understand the pathogenesis of *P. carinii* pneumonia, with the hope that we can use this information to control or prevent this disease.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000073-13

LBC: CCM

Title: Investigations of New Therapies in Septic Shock

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Charles Natanson, M.D.

Collaborators, Lab: Steven Banks (CCM, CC)
Peter Q. Eichacker, M.D. (CCM, CC)
Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Stephen S. Richmond (CCM, CC)
Steven Solomon, Ph.D. (CCM, CC)

Total Staff Years: 1

Human Research: Neither human cells nor tissues

Keywords: Septic Shock, New Therapies

Summary: Septic shock and related sequelae of infection (e.g., multiple organ system failure) are the most common cause of death in intensive care units. Deaths due to sepsis can occur in previously healthy individuals, in all age groups, and in a variety of common clinical settings. Some common predisposing conditions are premature neonates, previously healthy children with acquired infections (e.g., meningitis, pneumonia, upper respiratory infections), teenagers or young adults with trauma or cancer, and elderly patients with pneumonia or gall bladder disease. Half of all children or adults who acquire this septic shock die from the syndrome. Thus, septic shock, affecting young children and the elderly alike (even those without predisposing illness), is a common and important clinical problem with substantial mortality, which produces a great financial burden on society. Surprisingly little is known about the pathophysiology of this disease infection (organism virulence factors and toxins) and factors related to the host response (endogenous molecules that affect and modulate the inflammatory response). Successful treatment of the septic shock syndrome, which reduces morbidity and mortality, will result from curing the infection and interrupting the effects of these organisms and host mediators. The canine model of septic shock, using purpose-bred beagles, has successfully provided information on the pathophysiology and treatment of human disease. This model of acute and chronic infection simulates the course and cardiovascular changes seen routinely in children and adult humans with septic shock. Prior experiments using the model have established the role of specific bacteria (gram positive and gram negative), bacterial toxins (endotoxin), and host mediators to produce septic shock. Thus, the canine model has been highly successful in simulating the human disease and guiding therapy for humans. There are several therapies under investigation that might be effective in human septic shock. The canine model, which simulates the cardiovascular changes seen in children and adults with septic shock, is ideally suited for preclinical trials of these new therapies. The canine model allows properly controlled trials to evaluate therapeutic mechanisms and adverse effects of therapies, which are not always possible in human studies. We are evaluating or will evaluate the following therapies for septic shock in the canine model:superoxide dismutase; tyrosine kinase inhibitors; endotoxin precursors; bradykinin antagonists; dantrolene, soluble tumor necrosis factor receptor; antibodies to CD-18 receptors on white blood cells; ibuprofen; antibodies to human tumor necrosis factor receptor; granulocyte stimulating factor; antibodies to platelet activating factor; activated protein C; continuous arteriovenous hemofiltration; high-density lipoproteins; platelet activating factor; inhibitor antidiuretic hormone; and left ventricular assist devices.

LBC: CCM

Title: Effect of Nitric Oxide Synthase Inhibitors in *In Vivo* Tumor Necrosis Factor-induced Myocardial Depression

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Charles Natanson, M.D.

Collaborators, Lab: Steven Banks (CCM, CC)
Robert L. Danner, M.D. (CCM, CC)
Peter Q. Eichacker, M.D. (CCM, CC)
Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Stephen S. Richmond (CCM, CC)
Steven Solomon, Ph.D. (CCM, CC)

Total Staff Years: 1.5

Human Research: Neither human cells nor tissues

Keywords: Nitric Oxide Synthase Inhibitors, Tumor Necrosis Factor-induced Myocardial Depression

Summary: The present investigation has been undertaken to determine, *in vivo*, if nitric oxide is responsible for cytokine-induced myocardial depression. The negative ionotropic effects of cytokines on the heart are believed to be mediated by nitric oxide, Based on *in vitro* data. In isolated hamster cardiac papillary muscle, this negative ionotropic effect of cytokines can be blocked by N-G monomethyl-L-arginine (NMA), a nitric oxide synthase inhibitor. Because the *in vitro* data demonstrate that nitric oxide synthase inhibitors prevented tumor necrosis factor (TNF)-induced myocardial depression of rapid onset and reversal, we studied a low dose of recombinant human TNF challenge in canines. This TNF challenge produces significant, early, and short-lived myocardial depression (resolved by 24 hours). Surprisingly, we found that NMA did not prevent the early (up to 6 hours) deleterious effects of TNF on cardiac function. In fact, during this time period, TNF and NMA effects on all cardiac and hemodynamic parameters were additive (i.e., NMA did not block TNF effects). However, 24 hours after TNF infusion, NMA. did ameliorate the effects of TNF on some parameters such as acid-base derangements and decreases in mean arterial pressure and systemic vascular resistance. These data suggest that the early phase of TNF-induced cardiac and vascular abnormalities may not be related to nitric oxide production. However, some of the later effects of TNF may be related to the production of nitric oxide. Given the suggestive finding of a beneficial effect of NMA 24 hours post-TNF infusion, we evaluated the effects of nitric oxide inhibition in the setting of higher doses of TNF causing longer-lasting myocardial depression. Previous experiments using TNF challenges in canines suggest that this is a reasonable hypothesis (i.e., there may be two phases of cardiac injury). In canines, there is an early (less than 8 hours), dose-independent mechanism of myocardial depression and a late (greater than 24 hours), dose-dependent mechanism of myocardial depression. It was hypothesized that the inhibition of nitric oxide synthesis might not be advantageous early when myocardial depression is dose dependent. Therefore, we studied both prophylactic treatment (pretreatment) and therapeutic treatment with NMA (post-treatment after TNF challenge examining both early- and late-time points).

Treatment with NMA. lowered measures of nitric oxide production in both early- and late-time points. At early-time points, NMA given either therapeutically or prophylactically did not prevent the adverse effects of TNF. However, at 24 hours after reversal of the NMA. with l-arginine, the natural substrate for nitric oxide production, prophylactic NMA. ameliorated the decline in cardiac function seen with TNF challenge. These data suggest a dual effect of TNF on cardiac function. The early effect appears to be nitric oxide independent, while the later effect appears to be nitric oxide dependent. In future studies, we plan to confirm these findings in actual bacterial infection-induced myocardial depression models. NMA. is being used with cytokine therapy for cancer patients to inhibit cardiovascular toxicity, and studies are planned in AIDS patients to do the same. These studies will help determine the advisability of this approach.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000134-08

LBC: CCM

Title: Effect of Reconstituted High-density Lipoproteins in a Canine Model of Septic Shock

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Charles Natanson, M.D.

Collaborators, Lab: Robert L. Danner, M.D. (CCM, CC)
Peter Q. Eichacker, M.D. (CCM, CC)

Total Staff Years: 2.5

Human Research: Neither human cells nor tissues

Keywords: Septic Shock, Canine Model, High-density Lipoproteins

Summary: This project has been completed and a manuscript has been published in the *Journal of Pharmacology and Experimental Therapeutic*s 1999;288(1):107-113.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000146-08

LBC: CCM

Title: Characterization of Immune Responses during *Pneumocystis carinii* Pneumonia

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Kovacs, M.D.

Collaborators, Lab: Lisa Bishop (CCM, CC)
Geetha Kutty (CCM, CC)

Collaborator, NIH: Irini Sereti (DIR, NIAID)

Total Staff Years: .5

Human Research: Neither human cells nor tissues

Keywords: Immune Responses, *Pneumocystis Carinii* Pneumonia

Summary: *Pneumocystis carinii* is a major pathogen of patients with HIV infection. The immune responses to *P. carinii* are poorly understood, but cytokines may play a role in both clearing *P. carinii* infection and in the hypoxia associated with *P. carinii* pneumonia (PCP) that may be exacerbated following initiation of therapy. We are using the scid mouse model, as well as other immunodeficient mice, to further evaluate the role of individual cytokines and other immunoregulatory molecules in modulating *P. carinii* infection. We are in the process of developing techniques that will allow assessment of which cytokines are produced in response to *P. carinii* antigens. We have also developed a real-time polymerase chain reaction assay for quantitative PCP over a wide dynamic range and will be examining PCP infection in healthy animals to better understand immune responses in the normal host. It is hoped that these studies will provide insights into the role of cytokines in *P. carinii* pneumonia and may provide mechanisms for increasing clearance of *P. carinii* or decreasing the inflammation that may be causing hypoxia.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000149-08

LBC: CCM

Title: Study of Control of Cytosolic Phospholipase A2 Gene Expression in Airway Epithelial Cells

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James H. Shelhamer, M.D.

Collaborator, Lab: Rafal Pawliczak, M.D., Ph.D. (CCM, CC)

Total Staff Years: 2

Human Research: Human cells or tissues

Keywords: Airway Epithelial Cells

Summary: The 5' promoter region of the cytosolic phospholipase A2 (cPLA2) gene has been cloned and sequenced. The promoter for the cPLA2 gene does not have a TATA box but is inducible. Reporter genes with inserts extending from the 5' portion of the promoter region to the first intron have been made, and reporter genes with mutations in a putative initiator region have been utilized to characterize the control mechanisms important in expression of this gene. Sequences important in control of transcription have been identified. A minimal promoter sequence has been identified. Nucleotides within the initiator region, which are critical to basal transcription, are under study. An initiator element at the transcription start site is critical for initiation of transcription. Further, a sequence of nucleotides 30 to 36 bases 5' to the transcription start site is critical to the initiation function. Two series of nucleotide repeats have also been identified. These repeats appear to act to downregulate basal transcriptional activity as measured by mutation and deletion reporter gene constructs.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000155-06

LBC: CCM

Title: Study of Salvage Regimens for Patients with HIV Infection Demonstrating Virologic Failure with Licensed Protease Inhibitors

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Henry Masur, M.D.

Collaborators, NIH: Judith Falloon, M.D. (CMRS, NIAID)
Henry Lane, M.D. (CMRS, NIAID)
Susan Vogel, R.N. (CMRS, NIAID)

Total Staff Years: 1

Human Research: Human subject research: cells or tissues

Keywords: HIV Infection, Virologic Failure, Protease Inhibitors

Summary: Highly active antiretroviral therapy has revolutionized the therapy of HIV disease, yet a substantial fraction of patients either never respond or lose virologic control during the first 3 years of therapy. In this study, patients who have failed HAART were treated with three agents. The study had two major goals: (1) to determine the response rate to these agents, and (2) to determine what clinical and laboratory parameters, especially genotypic and phenotypic analysis, are useful for predicting response. Accrual of 98 patients has been completed; all have reached 48 weeks of followup. Results show that phenotypic assays are useful for predicting response to salvage regimens and that toxicity is common, especially for patients with high viral loads. Genotypic assays were also useful for predicting response, but their interpretation is much more complex than interpretation for phenotypic assays. This manuscript will be published in *AIDS* in 2002.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000164-06

LBC: CCM

Title: A Controlled Trial of Tyrosine Kinase Inhibitors in a Canine Model of Septic Shock

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Charles Natanson, M.D.

Collaborators, Lab: Robert L. Danner, M.D. (CCM, CC)
Peter Q. Eichacker, M.D. (CCM, CC)
Michael A. Solomon, M.D. (CCM, CC)
Steven Solomon, Ph.D. (CCM, CC)

Total Staff Years: 1

Human Research: Neither human cells nor tissues

Keywords: Tyrosine Kinase Inhibitors, Canine Model, Septic Shock

Summary: Septic shock appears to result from excessive release of cytokines (e.g., tumor necrosis factor-α [TNF-α], IL-2, etc.) and other pro-inflammatory substances (e.g., nitric oxide [NO]) from cells of the monocyte/macrophage lineage in response to infection or lipopolysaccharide (LPS) administration. The production of these cytokines, as well as their action, is mediated by signal transduction events, which induce protein tyrosine phosphorylation. Theoretically, inhibition of protein tyrosine phosphorylation may be beneficial in sepsis. These compounds would block the potentially high cytokine production, which is dependent on tyrosine phosphorylation. These protein kinase inhibitors would block activation or production of cytokinase by bacterial products and the effects of cytokines on target cells. Tyrphostins AG 126 and AG 556 are both protein kinase inhibitors and have been shown to improve outcome in small animal models during both LPS and live bacterial challenge. Further, both AG 126 and AG 556 have been shown to inhibit LPS-induced TNF production from dog peripheral blood mononuclear cells, *in vitro*. Studies in large animal models are needed before we can begin human clinical trials to establish efficacy and safety of these compounds. In collaboration with Dr. Novogrodsky and his colleagues, we evaluated AG 126 and AG 556 in our canine peritonitis model. In a controlled clinical trial in 100 animals over 6 months, AG 556, but not AG 126, significantly improved survival and prevented multiorgan failure during canine septic shock. This therapeutic agent (AG556) is proceeding to human clinical trials.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000165-06

LBC: CCM

Title: Retrospective Assessment of Pulmonary Infection in Patients with Chronic Granulomatous Disease

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Frederick P. Ognibene, M.D.

Collaborator, Lab: Mark T. Gladwin, M.D. (CCM, CC)

Collaborators, NIH: John I. Gallin, M.D. (CCM, CC)
Steven M. Holland, M.D. (LHD, NIAID)

Collaborator, Extramural: Anthony Slonim, M.D., Critical Care Medicine
Children's National Medical Center

Total Staff Years: .2

Human Research: Human subject research

Keywords: Chronic Granulomatous Disease, Pulmonary Infection

Summary: Patients with chronic granulomatous disease (CGD) frequently develop pulmonary infections. In many patients with CGD, an accurate infectious diagnosis is difficult to establish due to the scarcity of organisms in pathologic specimens. This study assesses, in a retrospective manner, all procedural as well as diagnostic data in patients with CGD and pulmonary disease. The study assesses the utility of sputum, bronchoscopy (lavage and transbronchial biopsy), CTT-guided transthoracic needle aspiration, and open-lung biopsy in establishing either a definitive or presumptive pulmonary diagnosis. Data collection for the research project has been completed. The data have been entered into a comprehensive database in order to facilitate manipulation and analysis and are now being reviewed. There are a total of 50 patients for whom the clinical characteristics, and the radiologic, microbiologic, and cytopathologic results of pulmonary infections are available. To date, the microbiologic and radiologic data have been catalogued, including over 600 microbiologic procedures and 1,800 radiologic procedures. At the completion of the project, a diagnostic, evidence-based algorithm will be available to aid in the diagnosis of pulmonary infections in patients with CGD.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000171-06

LBC: CCM

Title: Inflammatory Responses to Bronchial Endotoxin Instillation in Humans

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Anthony F. Suffredini, M.D.

Total Staff Years: 1.75

Human Research: Human subject research

Keywords: Bronchial Endotoxin

Summary: Administration of endotoxin to humans allows a unique way to evaluate the early inflammatory reactions that occur during infection. Characterizing these responses and the mechanisms that control them is important because these inflammatory responses contribute to the development of septic shock and organ failure. Under protocol 92-CC-0141, the effects of direct instillation of endotoxin into lung subsegments will be evaluated. Sequential bronchoalveolar lavage will be performed at 2, 6, 24, 48, or 72 hours after endotoxin instillation. Analyses will include the following: bronchoalveolar lavage for acute-phase cytokines; flow cytometry of neutrophils and lymphocyte subpopulations; and systemic and inflammatory effects including acute-phase cytokine release, recruitment of cells from the marrow, and the initiation of acute-phase protein release. An *in vitro* bilayer model of the alveolar blood interface has been designed to facilitate discovery of mechanisms that recruit inflammatory cells to the lung. These observations will be useful in defining important events in the initiation and resolution of acute lung inflammation to bacterial endotoxin.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000182-06

LBC: CCM

Title: Study of Control of Cytosolic Phospholipase A2 Activity

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James H. Shelhamer, M.D.

Collaborator, Lab: James Copeland, M.D., Ph.D. (CCM, CC)

Total Staff Years: 1

Human Research: Human cells or tissues

Keywords: Cytosolic Phospholipase A2

Summary: The activity of cytosolic phospholipase A2 (cPLA2) may be altered by calcium or by phosphorylation of serines in the cPLA2 molecule. A dual hybridization system in yeast was used to identify protein-protein interactions that might also be involved in the modulation of cPLA2 activity. Using this system, a member of the S-100 family of protein was identified as interacting with cPLA2. Ongoing studies include production of recombinant protein and modulation of enzyme function by oxidant molecules.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000184-05

LBC: CCM

Title: Influence of Previous Infection on the Host Defense Effects of Granulocyte Colony-stimulating Factor

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Peter Q. Eichacker, M.D.

Supervisor of Record: Henry Masur, M.D.

Collaborator, Lab: Xiaolin Cui, M.D. (CCM, CC)

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Keywords: Sepsis, Granulocyte Colony-stimulating Factor

Summary: Studies suggest that the production of endogenous anti-inflammatory agents during sepsis reduces host defense and predisposes the host to subsequent infection. Whether such a state of immunosuppression during sepsis actually exists or is reversible with the administration of pro-inflammatory agents is unclear. However, the use of recombinant granulocyte colony-stimulating factor (G-CSF) to augment host defense following the onset of sepsis in critically ill patients has been proposed. Using a rat model, we plan to determine whether an initial episode of infection and sepsis augments the host-defense effects of G-CSF during a subsequent episode.

LBC: CCM

Title: Reactive Oxygen Species Signaling by Endothelial Nitric Oxide Synthase

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Robert L. Danner, M.D.

Collaborators, Lab: Robert Ashe, B.A. (CCM, CC)
Ana del P. Cintron, M.T. (ASCP) (CCM, CC)
Xiaolin Cui, M.D. (CCM, CC)
Penglin Ma, M.D. (CCM, CC)

Total Staff Years: 4

Human Research: Neither human cells nor tissues

Keywords: Nitric Oxide Synthases, Nitric Oxide

Summary: Nitric oxide synthases (NOSs), the enzymes responsible for nitric oxide (NO) production from the substrate L-arginine, are also NADPH oxidases. In cell-free systems, some of these enzymes have been shown to produce reactive oxygen species such as superoxide. In this investigation, we transfected monoblastoid U937 cells with human endothelial NOS (eNOS) and found that TNFalpha production was increased but that this effect was not related to NO production. Further work found that eNOS upregulates TNFa by producing a reactive oxygen species (ROS), *(J Biol Chem*, 2000). Recent experiments have demonstrated that eNOS upregulation of TNFalpha occurs through superoxide-dependent activation of p44/42 mitogen-activated protein kinase (*Am J Physiol Cell Physiol*, 2001). Future work will focus on the switching mechanisms that regulate eNOS to produce either NO or ROS. A closely related effort will examine eNOS modulation of inflammatory responses in endothelial cells and the relative roles played by NO and ROS.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000189-04

LBC: CCM

Title: Study to Assess the Utility of Oral Washes to Diagnose Pneumocystis Pneumonia

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Henry Masur, M.D.

Collaborator, Lab: Barbara K. Hahn (CCM, CC)

Collaborators, NIH: Joseph A. Kovacs, M.D. (CCM, CC)
Steven Fischer, M.D., Ph.D. (DLM, CC)
Vee J. Gill, Ph.D. (MICRO, CC)
Sheng-ning Huang (CCM, CC)
Cliff Lane, M.D. (NIAID)
Jodie M. Parker (CC)

Collaborators, Extramural: Laurence Huang, M.D., University of California–San Francisco
Daniel Lucey, M.D., Washington Hospital Center

Total Staff Years: 1

Human Research: Human subject research: Minors

Keywords: Oral Washes, Pneumocystis

Summary: This study is part of a 15-year project to develop less invasive methods to diagnose pneumocystis pneumonia and to predict responses to therapy. Oral washes, induced sputum, and bronchalveolar lavage are being collected from patients with immunosuppressive diseases and respiratory syndromes. Samples for control patients are being collected as well. First, a polymerase chain reaction (PCR) technique using a unique major surface glycoprotein primer is being used in conjunction with a published primer to develop a method adaptable to clinical laboratories that is highly specific and sensitive. It is hoped that oral wash can replace sputum as the sample of choice. Second, mutations associated with drug resistance are being assessed in all organisms identified to determine the epidemiology and clinical importance of such mutations. Third, markers of strain variation are being assessed to elucidate pathogen epidemiology. Specimens are being obtained from NIH and the Washington Hospital Center. Results from 30 patients with morphologic evidence of PCP and 250 smear-negative immunosuppressed patients show that the oral wash has a 70 percent positive-predictive value and a 99 percent negative-predictive value. The oral wash, tested by PCR, is a useful screening test for PCP. This will be published in the *Journal of Infectious Diseases.* A quantitative assay has been developed to increase the positive-predictive value of this test. This technique (*Journal of Clinical Microbiology,* in press) separates colonized patients from those with disease. A prospective study assessing this quantitative test is in progress in collaboration with UCSF.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000190-04

LBC: CCM

Title: Physiologic Effects of Inhaled Nitric Oxide, Nitroglycerin, and Placebo in Study Subjects with Sickle Cell Anemia

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Mark T. Gladwin, M.D.

Supervisor of Record: Frederick P. Ognibene, M.D.

Collaborator, Lab: James H. Shelhamer, M.D. (CCM, CC)

Collaborators, NIH: Alan N. Schechter, M.D. (LCB, NIDDK)
Richard O. Cannon, M.D. (CB, NHLBI)
Constance T. Noguchi, Ph.D. (MCB, LCB, NIDDK)
Griffin P. Rodgers, M.D. (MCHB, NIDDK)

Total Staff Years: .5

Human Research: Human subject research

Keywords: Sickle Cell Anemia, Nitric Oxide, Nitroglycerin, Hemoglobin, Blood Flow

Summary: Sickle cell anemia is an autosomal recessive disorder and the most common genetic disease affecting African Americans. Approximately 0.15 percent of African Americans are homozygous for sickle cell disease, and 8 percent have sickle cell trait. Acute pain crisis and acute chest syndrome (ACS) are common complications of sickle cell anemia. Inhaled nitric oxide (NO) has been proposed as a possible therapy for ACS. Anecdotally, NO has been described to rapidly improve the hypoxemia and the clinical course of ACS. Furthermore, a number of recent studies have suggested that NO may have a favorable impact on sickle hemoglobin at the molecular level and could improve the abnormal microvascular perfusion that is characteristic of sickle cell anemia. This clinical trial was designed to evaluate the physiologic and molecular effects of inhaled NO and a currently available, safe, FDA-approved medication, nitroglycerin, which is a nitric oxide donor (i.e., a source of NO after metabolism in the body), in study subjects with and without sickle cell anemia. Whole blood was analyzed to characterize the metabolism of NO and NO donors, the molecular interactions between hemoglobin and NO, the duration of effect of these therapies on hemoglobin oxygen affinity, and other properties of the erythrocyte and intracellular hemoglobin (including the solubility of deoxy sickle hemoglobin). We found that during NO inhalation at 80 ppm, NO binds to the heme of hemoglobin and is delivered to the peripheral circulation. The amount delivered is sufficient to restore regional blood flow to the forearm during NO synthase inhibition (measured by strain-gauge plethysmography). This may prove an effective therapy to increase regional blood flow during sickle cell pain crisis and after vascular procedures such as angioplasty. We also characterized the effect of NO delivery on microvascular perfusion in study subjects with and without sickle cell anemia by magnetic resonance imaging (MRI) of lower extremity skeletal muscle enhancement during first passage of intravenously injected gadolinium contrast. Perfusion measurements were paired with 31-phosphorus magnetic resonance spectroscopy (31-P-MRS) study of the concentration of muscle high-energy phosphate compounds. We were unable to appreciate changes in blood flow in our pilot study using this imaging modality. This ongoing project

will allow three major assessments: (1) the characterization of the microvascular perfusion at rest and during exercise in study subjects with sickle cell anemia, (2) the effects of NO on red cell and hemoglobin function and skeletal muscle perfusion in normal study subjects (without sickle cell anemia), and (3) the effects of NO on red cell and hemoglobin function and skeletal muscle perfusion in study subjects with sickle cell anemia. Our hypothesis is that one or more of these effects could be of potential therapeutic benefit to sickle cell anemia patients.

LBC: CCM

Title: Studies of Lymphocyte Kinetics in Healthy and HIV-infected Patients

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Kovacs, M.D.

Collaborators, Lab: Grace G. Kelly, R.N. (CCM, CC)
Henry Masur, M.D. (CCM, CC)

Collaborators, NIH: Richard T. Davey, M.D. (CRS, NIAID)
Dimiter S. Dimitrov, Ph.D. (NCI)
Judith Falloon, M.D. (CMRS, NIAID)
Betsey R. Herpin (CMRS, NIAID)
Henry Lane, M.D. (OCR, NIAID)
Susan Leitman, M.D. (DTM, CC)
Michael Polis, M.D. (CMRS, NIAID)
Douglas J. Schwartzentruber, M.D. (SB, NCI)
Igor A. Sidorov (NCI)
Jorge L. Tavel, M.D. (DIR, NIAID)

Collaborators, Extramural: Joseph Aldesberger, Ph.D., SAIC
Michael Baseler, Ph.D., SAIC
Richard Lempicki, Ph.D., SAIC

Total Staff Years: .5

Human Research: Human subject research

Keywords: Lymphocyte Kinetics, HIV

Summary: Understanding the rate of lymphocyte replication and destruction in HIV-infected patients, as well as the effects of therapy on lymphocyte replication, should lead to a better understanding of the mechanisms behind the immunodeficiency induced by HIV. Little is known about the replication rate in healthy and HIV-infected patients. Two approaches are being used to address this issue. (1) Healthy and HIV-infected patients will receive up to 5 days of continuous infusions with [6,6-2H2]-glucose, a nonradioactive, stable isotope of glucose that is safe to administer. The deuterium is incorporated into DNA via metabolism of glucose to ribose and incorporation into nucleotides. The rate of incorporation can be measured in subpopulations of cells to determine the rate of replication of those cells, and the rate of loss of the incorporated deuterium can be used to examine the turnover rate of the replicated cells. (2) HIV-infected patients will receive bromodeoxyuridine (BrDU; 200 mg/m2), an analogue of thymidine. BrDU is incorporated into DNA and incorporation can be measured using an anti-BrDU monoclonal antibody. By FACS analysis, both surface markers and BrDU can be measured. Thus, FACS analysis can be used to directly measure subpopulations of cells that have replicated. To date, 35 patients have been enrolled in these studies. Techniques for measuring incorporation have been developed and validated for both methods. Studies with BrDU have identified two populations of proliferating cells, one with a rapid turnover and another with a slow turnover. The size of the rapidly proliferating pool, but not the slowly proliferating pool, is directly related to the log viral load, suggesting that HIV drives cells to enter the rapidly proliferating pool. Studies are ongoing to follow up on these observations and to evaluate lymphocyte replication in other settings. These two approaches should provide information about lymphocyte kinetics that will have relevance to HIV infection and other disease states.

LBC: CCM

Title: Molecular Studies of Human *Pneumocystis carinii*

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Kovacs, M.D.

Collaborators, Lab: Geetha Kutty (CCM, CC)
Ma Liang, M.D. (CCM, CC)
Henry Masur, M.D. (CCM, CC)

Collaborators, NIH: Steven Fischer, M.D., Ph.D. (DLM, CC)
Vee J. Gill, Ph.D. (MICRO, CC)

Collaborator, Extramural: Laurence Huang, M.D., University of California–San Francisco

Total Staff Years: 1.5

Human Research: Human cells or tissues

Keywords: *Pneumocystis carinii*

Summary: *Pneumocystis carinii* infections remain common in HIV-infected patients despite the broad use of highly active antiretroviral therapies and prophylactic regimens. Studies of human *P. carinii* are focusing on two areas: diagnosis and evaluation for potential resistance to therapy. To try to develop highly sensitive, noninvasive diagnostic methods, we have been evaluating polymerase chain reaction (PCR) using primers based on the major surface glycoprotein (MSG) genes of human *P. carinii*. This is a family of genes that are closely related and that encode an important surface protein of *P. carinii*. PCR using primers based on this gene is potentially a highly sensitive method, because this is a multicopy gene (estimated at greater than 100 copies/genome). We have been evaluating the diagnostic potential using a conserved region of the gene family. Our studies have shown that the sensitivity of MSG-based primers is greater than that of previously utilized primers. We are currently evaluating these primers prospectively in collaboration with the Microbiology Department. Because human *P. carinii* cannot be cultured, we cannot directly determine if resistance to commonly used therapeutic agents is developing. However, molecular techniques can be used to identify mutations that may confer resistance in genes that are targets of therapeutic agents. The most commonly used agent to treat *P. carinii* pneumonia is the combination of trimethoprim, which targets dihydrofolate reductase (DHFR), and sulfamethoxazole, which targets dihydropteroate synthase (DHPS). We have cloned the human *P. carinii* DHFR gene and have examined (by PCR and sequencing) the *P. carinii* DHFR and DHPS genes of a variety of human isolates from patients with *P. carinii* pneumonia. DHPS mutations were found in about one-third of patients, while no mutations have been found to date in the DHFR gene. We have also expressed recombinant human *P. carinii* DHFR and characterized the kinetics of this enzyme. Over the past year, we have developed a rapid method for detection of DHPS mutations using SSCP (single strand conformational polymorphisms) and have examined a large number of samples for DHPS mutations, including organisms obtained from an Italian cohort. We are also evaluating a new typing technique using tandem repeats that occur in an intron of the MSG gene. These studies should provide improved diagnostic methods for PCP and insights into the reasons for therapy or prophylaxis failures.

LBC: CCM

Title: Tyrphostin AG 556 Therapy Adjusted to Severity of Illness of New Therapies in Septic Shock

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Charles Natanson, M.D.

Collaborators, Lab: Steven Banks (CCM, CC)
Peter Q. Eichacker, M.D. (CCM, CC)
Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Stephen S. Richmond (CCM, CC)
Steven Solomon, Ph.D. (CCM, CC)

Total Staff Years: 3

Human Research: Neither human cells nor tissues

Keywords: Septic Shock, Tryphostin AG556

Summary: Septic shock appears to result from excessive release of cytokines (e.g., tumor necrosis factor-α [TNF-α], IL-2, etc.) and other pro-inflammatory substances (e.g., nitric oxide [NO]) from cells of the monocyte/macrophage lineage in response to infection or lipopolysaccharide (LPS) administration. The production of these cytokines, and their action, is mediated by signal transduction events that induce protein tyrosine phosphorylation. Theoretically, inhibition of protein tyrosine phosphorylation may be beneficial in sepsis. These compounds would block the potentially high cytokine production that is dependent on tyrosine phosphorylation. These protein kinase inhibitors would block both activation and production of cytokines by bacterial products and the effects of cytokines on target cells. Tyrphostins AG 126 and AG 556 are both protein kinase inhibitors and have been shown to improve outcome in small animal models during both LPS and live bacterial challenge. Further, both AG 126 and AG 556 have been shown to inhibit LPS-induced TNF production from dog peripheral blood mononuclear cells, *in vitro*. In collaboration with Dr. Novogrodsky and his colleagues, we evaluated AG 126 and AG 556 in our canine peritonitis model. In a controlled clinical trial in 100 animals over 6 months, AG 556 but not AG 126 significantly improved survival and prevented multiorgan failure during canine septic shock. Recent analysis of animal experimental data suggests that the effect of anti-inflammatory agents is dependent in part on the underlying infectious burden of the animal. It appears that studies in which controls exhibited high mortality showed improved survival in response to anti-inflammatory therapy. Conversely, studies in which controls exhibited lower mortality suggested that anti-inflammatory agents had no benefit, and possibly some harm. Therefore, it is possible that the reason that human clinical trials in sepsis have shown no benefit is that the anti-inflammatory agents have been given to individuals with varying degrees of illness, and that a subgroup of patients with higher burden of illness might be helped by anti-inflammatory therapy. This study is designed to examine the effect of titrating AG 556 to the severity of illness in canines infected with high- and low-infectious burdens. In our canine model of peritonitis, cohorts of animals with either high or low burdens of *E. coli* peritonitis clots will be studied. We will compare the efficacy with standard dose 2.5 mg/kg AG 556 to placebo, to titrated dosing 1 mg/kg and then 1 or 4 mg/kg depending upon the blood pressure of animals at the 6-hour time point. This study is the first study in an animal model to examine whether the utility of anti-inflammatory therapy is dependent upon the burden of infectious agent and has potential implication for human clinical trials of anti-inflammatory agents in sepsis.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001157-03

LBC: CCM

Title: Effects of Inhaled Nitric Oxide on Pulmonary Inflammatory Responses

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Anthony F. Suffredini, M.D.

Collaborators, Lab: Carmen Fiuza, M.D. (CCM, CC)
Mark T. Gladwin, M.D. (CCM, CC)
Margaret M. Tropea (CCM, CC)

Total Staff Years: 1.75

Human Research: Human subject research

Keywords: Nitric Oxide, Pulmonary Inflammatory Responses

Summary: Inhaled nitric oxide (NO) diminishes inflammatory responses *in vitro* and in some animal models of lung inflammation. We are studying the mechanisms involved in NO modulation of local pulmonary inflammation in humans. Evidence suggests that NO can modulate the inflammatory response in experimental lung inflammation. Nitric oxide donors inhibit inflammatory cytokine production by human alveolar macrophages *in vitro*, prevent IL-1 induced neutrophil accumulation and edema in isolated rat lungs, and block increases in pulmonary lavage neutrophils, protein, and lung myeloperoxidase content in septic swine. Only limited data are available in humans treated with inhaled NO for acute lung injury. After 4 days of inhaled NO, patients had a reduction of BAL neutrophil spontaneous H2O2 production, CD11b/CD18 expression, and IL-6 and IL-8 in BAL fluid compared with patients who did not receive inhaled NO. Nitric oxide remains under investigation for adjunctive therapy for acute lung injury. We are evaluating the ability of NO to alter the inflammatory response associated with segmental endotoxin instillation. Twenty-four volunteers will be studied in a randomized fashion. An initial pilot study will be performed in eight subjects challenged with bronchial endotoxin instillation. Following the endotoxin instillation, four subjects will breathe NO (40 ppm), delivered by an anesthesia nonrebreathing face mask with a reservoir bag, and four subjects will breathe room air through a similar mask. The subjects will breathe through the circuit for 6 hours. The lavage cells will be studied using cell culture, functional studies, surface markers, and intracellular cytokines with flow cytometry and mRNA expression. The lavage supernatant will be evaluated for various inflammatory mediators and markers of inflammatory cell activation. Sequential blood samples will be obtained for total leukocyte counts, as well as plasma levels of inflammatory mediators.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001158-03

LBC: CCM

Title: Role of Nitric Oxide in Regulating Inflammation and Gene Expression

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Robert L. Danner, M.D.

Collaborators, Lab: Xiaolin Cui, M.D. (CCM, CC)
Penglin Ma, M.D. (CCM, CC)
Jianhua Zhang, Ph.D. (CCM, CC)

Collaborator, NIH: Harry L. Malech, M.D. (LHD, NIAID)

Total Staff Years: 4

Human Research: Human cells or tissues

Keywords: Nitric Oxide

Summary: Nitric oxide (NO) is an important intercellular and intracellular messenger implicated in the pathogenesis of septic shock. Inhibition of NO synthase is under investigation as a treatment for hypotension in septic shock. In addition to the vasodilating effect of NO, this messenger also has effects on platelets and immune cells. In this investigation, we are examining the role of the NO pathway as a modulator of immune cell function and gene expression. We have been unable to create conditions under which human phagocytes, in particular neutrophils, endogenously produce NO (*J Immunol*: 1825, 1994). Therefore, the ability of NO produced by other cells, such as endothelium and epithelium, to alter the function of human phagocytes is being explored. “We have confirmed that NO regulates cytokine production using a U937 monocytic cell line transfected to express murine-inducible NO synthase” (*Blood*: 1160, 1997). Further investigation of this effect has resulted in the description of a cGMP-independent signaling pathway for NO (*J Biol Chem*: 5959, 1997). We have found that in addition to upregulating TNFα production (*J Immunol*: 4102, 1994) NO modulates IL-8 message transcription and release in human neutrophil preparations. However, contrary to other reports, NO does not directly alter neutrophil chemotaxis (*J Infect Dis*: 116, 1998). More recent work has identified an NO-response element in the TNFα promoter (*J Biol Chem*, 1999). Recent experiments have generalized the role of this putative NO-response element to several unrelated promoters. Further, the importance of sequences flanking this NO-response element to its function is being investigated. Work with the IL-8 promoter suggests that this chemokine is regulated by NO through a mechanism that is also cGMP-independent but distinct from the pathway that regulates TNFα. In a new phase of this project, expression microarrays will be used to define larger sets of genes regulated by NO and to dissect out the underlying mechanisms by which the regulation occurs.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001159-03

LBC: CCM

Title: Magnetic Resonance Imagery Study of Avascular Necrosis of the Hip in Asymptomatic HIV-infected Patients

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Henry Masur, M.D.

Collaborators, Lab: Grace G. Kelly, R.N. (CCM, CC)
Joseph A. Kovacs, M.D. (CCM, CC)

Collaborators, NIH: Richard T. Davey, M.D. (CRS, NIAID)
Judith Falloon, M.D. (CMRS, NIAID)
Lynn H. Gerber, M.D. (RM.D., CC)
Galen O. Joe (CC)
Elizabeth C. Jones (DDR, CC)
Cliff Lane, M.D. (NIAID)
Joann Mican, M.D. (DIR, NIAID)
Michael Polis, M.D. (CMRS, NIAID)
Margaret E. Rick, M.D. (HEME, CC)

Total Staff Years: .5

Human Research: Human subject research

Keywords: MRI, Magnetic Resonance Imaging, HIV

Summary: Avascular necrosis (AVN) of the hip has occasionally been reported in the setting of HIV infection. After we diagnosed AVN in two patients in May 1999, we became concerned that HIV-infected patients may be at higher risk for developing AVN than previously recognized. To examine this, we undertook a magnetic resonance imaging (MRI)-based study of asymptomatic HIV-infected patients to determine the prevalence of hip AVN in our clinic population. Fifteen of 339 (4.4 percent) patients had evidence of AVN by MRI: six had bilateral and nine had unilateral involvement. None of 118 HIV-negative volunteers had MRI evidence of AVN. Prospectively performed physical examinations did not distinguish HIV-infected patients with AVN from those without. Patients with osteonecrosis were more likely than patients without osteonecrosis to have used corticosteroids, lipid-lowering agents, and testosterone and were more likely to routinely exercise by bodybuilding. Thus, HIV-1 infection should be included among medical conditions that predispose to the development of osteonecrosis. Long-term followup of this cohort is under way to determine the natural history of symptomatic and asymptomatic lesions, and the incidence of new lesions.

LBC: CCM

Title: Study of the Control of p11 Protein Production

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James H. Shelhamer, M.D.

Collaborators, Lab: Mark Cowan, M.D. (CCM, CC)
Xiuli Huang, M.D. (CCM, CC)

Total Staff Years: 2

Human Research: Human cells or tissues

Keywords: p11 Protein Production

Summary: p11 is a protein that can bind to and inhibit cytosolic phospholipase A2. Modulation of p11 levels might provide a way to control a variety of cellular functions. Control of p11 has been studied at the protein and mRNA level. The p11 5' promoter has been cloned, sequenced, and characterized. p11 protein production has been studied in response to dexamethasone and to retonoic acid. The effect of cytokine and growth factor stimulation of epithelial cells on p11 production is also being studied. Two manuscripts have been published and two are in preparation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001163-02

LBC: CCM

Title: Endothelial Cell Response to Oxidative Stress

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James H. Shelhamer, M.D.

Collaborator, Lab: Uday Nanavaty, M.D. (CCM, CC)

Total Staff Years: 1

Human Research: Human cells or tissues

Keywords: Endothelial Cell Response, Oxidative Stress

Summary: The response of human lung epithelial cells and endothelial cells to oxidative stress is being studied at the level of cellular function and gene expression. Signal transduction pathways activated in response to oxidative stress and linked to these events are also under active investigation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001164-02

LBC: CCM

Title: Functional Genomics of Critical Illness

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Robert L. Danner, M.D.

Collaborators, Lab: Robert Ashe, B.A. (CC)
Ana del P. Cintron, M.T. (ASCP) (CC)
Peter Q. Eichacke, M.D. (CC)
Charles Natanson, M.D. (CC)
Zenaide Quezado, M.D. (CC)
James H. Shelhamer, M.D. (CC)
Michael A. Solomon, M.D. (CC)
Anthony F. Suffredini, M.D. (CC)
Jianhua Zhang, Ph.D. (CC)

Collaborator, NIH: Steven M. Holland, M.D. (LHD, NIAID)

Collaborators, Extramural: J. Perren Cobb, M.D., Washington University
D. Golenbach, M.D., Boston University

Total Staff Years: 10

Human Research: Human subject research: cells or tissues

Keywords: Critical Illness, Genomics

Summary: Critical illness syndromes—such as acute respiratory distress syndrome (ARDS), septic shock, myocardial depression, and multiple organ failure—all share the hypothesis that the host response plays a central pathogenic role. In this project, CCMD has established the infrastructure necessary to define these pathogenic host responses at the level of gene expression across thousands of mRNA transcripts, simultaneously. This technology will be used to create a large critical illness functional genomics database using *in vitro* models, small animal (rat and mouse) models, endotoxin-challenged volunteers, and, ultimately, critically ill patients. Preliminary work has identified more than 350 genes that are differentially regulated by the administration of endotoxin to healthy volunteers. Increasing bacterial doses in rats has shown that a number of genes are expressed in a grade manner that corresponds closely to infection severity.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001165-02

LBC:	CCM
T*itle:*	Influence of Site, Severity, and Type of Infection on the Effects of Endotoxin Analogue in Sepsis
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Peter Q. Eichacker, M.D.
Supervisor of Record:	Henry Masur, M.D.
Collaborator, Lab:	Steven Solomon, Ph.D. (CC)
Collaborators, Extramural:	Various collaborators Eisai Research Institute
Total Staff Years:	2
Human Research:	Neither human cells nor tissues
Keywords:	Sepsis, Endotoxin Analogue

Summary: Clinical experience with anti-inflammatory agents in patients with sepsis has been disappointing to date. We have found that several factors—such as the site, type, and severity of infection—have important influences on many of these agents. Developing new agents that are impacted minimally by such factors will increase the usefulness of this therapeutic approach. The role of endotoxin in the injury of sepsis is unclear. It is likely that under many circumstances it produces harmful effects. Targeting endotoxin rather than host mediators may be a more generally useful goal in sepsis. Antibodies against endotoxin have had little effect to date in sepsis because the toxic lipid portion of the molecule may be difficult to target. An alternative approach to neutralizing endotoxin uses analogue molecules, which competitively inhibit cell signaling by endotoxin. We are presently performing a series of studies investigating the influence of site severity and type of infection on a competitive inhibitor of endotoxin.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001167-02

LBC:	CCM
Title:	Influence of Site, Severity, and Type of Infection on the Effects of Tyrosine Kinase Inhibitor in Sepsis
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Peter Q. Eichacker, M.D.
Supervisor of Record:	Henry Masur, M.D.
Collaborators, Lab:	Charles Natanson, M.D. (CC) Steven Solomon, Ph.D. (CC)
Collaborators, Extramural:	Various collaborators SignalSite Incorporated
Total Staff Years:	2
Human Research:	Neither human cells nor tissues
Keywords:	Sepsis, Tyrosine Kinase Inhibitor

Summary: Clinical experience with anti-inflammatory agents in patients with sepsis has been disappointing to date. We have found that severity of infection has an important influence on many of these agents. Developing new agents that are impacted minimally by this factor will increase the usefulness of this therapeutic approach. We are presently performing a series of studies investigating the influence of severity of infection on a recently developed tyrosine kinase inhibitor. In early studies with this agent, severity of inflammation related to endotoxin challenge did little to influence the beneficial effects of this agent.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001168-02

LBC: CCM

Title: Gene Expression in Humans Challenged with Endotoxin

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Anthony F. Suffredini, M.D.

Collaborators, Lab: Ana del P. Cintron, M.T. (ASCP) (CCM, CC)
Robert L. Danner, M.D. (CCM, CC)
Carmen Fiuza, M.D. (CCM, CC)
Carolea V. Logun (CCM, CC)
James H. Shelhamer, M.D. (CCM, CC)
Margaret M. Tropea (CCM, CC)

Collaborator, NIH: Peter J. Munson, Ph.D. (MSCL, CIT)

Collaborator, Extramural: Lawrence Hunter, Ph.D., Department of Extramural Experimental Pharmacology, University of Colorado Health Science Center

Total Staff Years: 1.75

Human Research: Human subject research

Keywords: Endotoxin, Biomarkers

Summary: Reliable biomarkers are needed to identify patients with sepsis who will benefit from anti-inflammatory therapies. In addition, recent observations suggest that novel mediators (i.e., calcitonin precursors, high mobility group-1 protein) play an important role in the pathogenesis of sepsis. In order to better characterize and discover new mediators and mechanisms involved in sepsis, we are using a model of inflammation based on the administration of endotoxin, a bacterial wall component, to normal volunteers. By administering endotoxin, either intravenously or via intrabronchial instillation, we are able to study early inflammatory events that occur in the blood and in the local environment of the lung. Intravenous endotoxin results in a systemic inflammatory response, which is associated with the release of acute phase cytokines and activation of inflammatory cells and endothelium. Bronchial endotoxin instillation results in a localized neutrophil influx, increased permeability to protein, and acute inflammatory mediator release in the lung. The resolution of the inflammation in the lung is associated with apoptosis of neutrophils and a mononuclear cell influx over the following 48 hours. Under protocol 92-CC-0141, the effects of endotoxin on gene expression will be studied using peripheral blood mononuclear cells and, in separate studies, cells obtained with bronchoalveolar lavage from the lung. The temporal pattern of gene expression will be studied using cDNA microarrays. Spotted and oligonucleotide arrays will be performed on total mRNA extracted from these cells. These tools will be useful to study fundamental aspects of gene expression during exposure to bacterial products. It will provide one means of characterizing new mediators and mechanisms that are part of the acute-phase response to bacterial products.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001169-02

LBC: CCM

Title: Inflammatory Effects of High Mobility Group Protein 1

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Anthony F. Suffredini, M.D.

Collaborators, Lab: Sura W. Alsaaty (CCM, CC)
Carmen Fiuza, M.D. (CCM, CC)
James H. Shelhamer, M.D. (CCM, CC)
Margaret M. Tropea (CCM, CC)

Collaborator, NIH: Michael Bustin, Ph.D. (LMC, NCI)

Total Staff Years: 1.75

Human Research: Human cells or tissues

Keywords: Endotoxin, Gene Expression, High Mobility Group Protein-1, Receptor for Advanced Glycation End Products

Summary: High mobility group protein (HMG-1) is a nonhistone DNA binding protein that facilitates transcription. Recently, investigators have shown that HMG-1 has other roles that may be critical in the development of sepsis and septic shock. HMG-1 is released as a late mediator of sepsis (i.e., after 8 to 15 hours) from mononuclear cells stimulated with TNF, IL-1, or endotoxin. Detected in the blood of septic mice and in septic patients, HMG-1 worsens outcome when given to septic mice. It plays a role in migrating axons of neurons in the developing brain and activates plasminogen. Some of the actions are through the RAGE receptor (receptor for advanced glycation products), which plays a role in chronic inflammation in diabetes. This novel axis of inflammation remains to be characterized in human sepsis. In order to study the target cells and contribution of HMG-1 to acute human inflammation, we are producing recombinant human HMG-1 in a bacterial expression system and are using the protein to study inflammatory responses in endothelium and mononuclear cells, including alveolar macrophages. In addition, we are developing biologically active peptide fragments of the intact molecule in order to study structure function relationships. Cell lines and migrating human cells from humans challenged with endotoxin will be studied for the expression of RAGE and responses to HMG-1. HMG-1 will also be studied in blood and inflammatory lavage obtained from volunteers challenged with endotoxin (protocol 92-CC-0141). Oligonucleotide gene arrays will be used to study the inflammatory axis initiated by HMG-1 on target cells. These data should provide important new information regarding the role of HMG-1 in acute human inflammation to bacterial products.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001170-01

LBC: CCM

Title: Study of Antiretroviral Pharmacokinetics in Patients from Diverse Racial and Geographic Regions

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Henry Masur, M.D.

Collaborator, Lab: Joseph A. Kovacs, M.D. (CCM, CC)

Collaborators, NIH:
Robert Eisinger (OAR, OD)
Cliff Lane, M.D. (NIAID)
Scott Penzak, Pharm.D. (CC)
Thomas Quinn, M.D. (IMS, NIAID)
Jorge L Tavel, M.D. (DIR, NIAID)

Collaborators, Extramural:
Richard Guerrant, M.D., INMD Geographic Medical
University of Virginia
Steve Piscitelli, Pharm.D., Clinical Pharmacology
Virco Lab, Inc.

Total Staff Years: .5

Human Research: Human subject research

Keywords: HIV, Developing World, Pharmacokinetics

Summary: Many factors may alter pharmacokinetics, including cytochrome P450 phenotype, malnutrition, intestinal parasites, drug source (manufacturer), and drug storage conditions. In this study, *in vitro* assays of drug bioavailability will be performed using antiretroviral agents produced in several different countries. Pharmacokinetic profiles of several standard regimens will be assessed in several distinct geographic regions. If profiles are substantially different, studies will be performed to assess the cause, and whether dosage regimens different from those recommended in the United States might be appropriate to maximize efficacy and safety.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001171-01

LBC: CCM

Title: Expression Profiling in Acute Cardiac Allograft Rejection

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Michael A. Solomon, M.D.

Supervisor of Record: Robert L. Danner, M.D.

Collaborators, Lab: Charles Natanson, M.D. (CCM, CC)
Rajnish Prasad, M.D. (CCM, CC)

Collaborator, NIH: Peter J. Munson, Ph.D. (ABS, CIT)

Collaborators, Extramural: Lawrence Hunter Center for Computational Pharmacology
University of Colorado Health Sciences Center
Andrew J. Keller, M.D., Heart Transplant Program
INOVA Transplant Center

Total Staff Years: 1

Human Research: Human subject research: Minors
Human cells or tissues

Keywords: Heart transplantation, Acute Rejection, Functional Genomics

Summary: Acute cardiac allograft cellular rejection remains a significant source of morbidity and mortality within the first year after heart transplantation. In the first year after transplantation, nearly 63 percent of patients experience at least one episode of cardiac rejection, and approximately one-third of these patients will have multiple episodes. The clinical symptoms of acute cardiac rejection are relatively nonspecific (fatigue, dyspnea, low-grade fever). No non-invasive method exists for the diagnosis of acute cardiac rejection. Several methodologies have been studied without success, including electrocardiography, echocardiography, nuclear imaging, and phosphorus spectroscopy. The current gold standard for the diagnosis of acute cellular rejection remains right ventricular endomyocardial biopsy. We propose applying functional genomics to the study of acute cardiac allograft cellular rejection. We hypothesize that large-scale expression profiling of circulating peripheral blood mononuclear cells (predominantly T lymphocytes) will identify genes that can serve as reliable biomarkers of acute cardiac cellular rejection. In the initial bench phase of the project, peripheral blood mononuclear cells would be harvested from heart transplant recipients during periods of immunologic tolerance of the allograft (no rejection) and immunologic intolerance of the allograft (rejection) to determine whether unique gene expression patterns are associated with each state. In the latter phase of the project, we hope to translate these profiles into an acceptable bedside test for acute cardiac allograft cellular rejection. In addition to developing a biomarker approach to the diagnosis of rejection in cardiac transplant patients, expression profiling has the potential to identify immunoregulatory pathways that can serve as new targets for immunosuppressive therapy (rational drug development).

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001172-01

LBC: CCM

Title: Determinants of Cardiac Function in a Canine Model of Septic Shock

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Steven Solomon, Ph.D.

Supervisor of Record: Charles Natanson, M.D.

Collaborators, Lab: Peter Q. Eichacker, M.D. (CCM, CC)
Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Steve Richmond (CCM, CC)

Collaborators, NIH: Andrew E. Arai, M.D. (LCE, NHLBI)
Michael A. Solomon, M.D. (CCM, CC)

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Keywords: Sepsis, MRI, Cardiac Mechanics, Diastolic Function

Summary: The potentially reversible myocardial depression of sepsis is well documented in humans and animals by radionuclide scans and intravascular catheter techniques. The mechanism of sepsis-induced myocardial depression remains incompletely understood. Sepsis-induced myocardial dysfunction cannot be explained by inadequate myocardial oxygen supply or insufficient myocardial high-energy synthetic capabilities. Investigators have postulated a myocardial depressant factor of sepsis, but the mechanisms by which bacteria, their toxins, and host cytokines disturb normal cardiac function remain unknown. Pro-inflammatory mediators have been implicated in the pathogenesis of congestive heart failure and the myocardial depression of sepsis. There is also electron microscopic evidence of diffuse abnormalities of the cardiac microvasculature characterized by endothelial cell swelling and nonocclusive intravascular fibrin deposition in septic animals. One can postulate that bacterial toxins and the induced host pro-inflammatory response disrupt the integrity of the myocardial microvasculature and subsequently injure the myocytes, resulting in myocardial functional depression. As in congestive heart failure, the heart adapts and maintains stroke volume through a remodeling mechanism, resulting in a reversible ventricular dilatation. The concept of ventricular dilatation in sepsis remains controversial. Sepsis studies using echocardiography to assess ventricular volumes have confirmed in humans and animals the depression of LV ejection fraction but not the LV dilatation. The purpose of this study is to better define systolic and diastolic abnormalities of the heart during sepsis and to determine if the sepsis-induced, pro-inflammatory response results in a cardiac microvascular injury that can lead to myocardial functional depression. We will quantify the changes in cardiac function using both invasive hemodynamic measurements and noninvasive cardiac magnetic resonance imaging (MRI). The data from the invasive measurements will be correlated with the noninvasive MRI data in order to develop an approach suitable for future human studies. Furthermore, this study is designed to definitively determine if sepsis-induced, myocardial depression is associated with microvascular flow abnormalities and LV dilatation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001173-01

LBC: CCM

Title: Nitric Oxide for Patients with Sickle Cell Anemia and Pulmonary Hypertension

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Mark T. Gladwin, M.D.

Supervisor of Record: Frederick P. Ognibene, M.D.

Collaborators, NIH: R.O. Cannon, M.D. (NHLBI)
James S. Nichols (CACPS, CC)
Griffin P. Rodgers, M.D. (MCHB, NIDDK)
Vandana Y. Sachdev (CB, NHLBI)
Alan N. Schechter, M.D. (LCB, NIDDK)

Total Staff Years: 2

Human Research: Human subject research

Keywords: Nitric Oxide, Sickle Cell Anemia, Hemoglobin, Blood Flow, Pulmonary Hypertension

Summary: Sickle cell anemia is an autosomal recessive disorder and the most common genetic disease affecting African Americans. Approximately 0.15 percent of African Americans are homozygous for sickle cell disease, and 8 percent have sickle cell trait. Acute pain crisis, acute chest syndrome (ACS), and secondary pulmonary hypertension are common complications of sickle cell anemia. Inhaled nitric oxide (NO) has been proposed as a possible therapy for both primary and secondary pulmonary hypertension. Furthermore, a number of recent studies have suggested that NO may have a favorable impact on sickle red cells at the molecular level and could improve the abnormal microvascular perfusion that is characteristic of sickle cell anemia. This clinical trial is designed (1) to determine the pathophysiologic processes that are associated with and potentially contribute to secondary pulmonary hypertension in adult patients with sickle cell anemia; (2) to determine the relative acute vasodilatory effects of oxygen, intravenous prostacyclin, and inhaled nitric oxide on pulmonary artery pressures and other hemodynamic parameters in patients with secondary pulmonary hypertension and sickle cell anemia; and (3) to determine the effects of 2 months of inhaled nitric oxide on pulmonary artery pressures, other hemodynamic parameters, exercise tolerance, and symptoms in this patient population.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001174-01

LBC: CCM

Title: Prevalence and Prognosis of Pulmonary Hypertension in Adults with Sickle Cell Anemia

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Mark T. Gladwin, M.D.

Supervisor of Record: Frederick P. Ognibene, M.D.

Collaborators, NIH: Richard O. Cannon, M.D. (CB, NHLBI)
James S. Nichols (CACPS, CC)
Vandana Y. Sachdev (CB, NHLBI)
Alan N. Schechter, M.D. (LCB, NIDDK)

Total Staff Years: 2

Human Research: Human subject research

Keywords: Nitric Oxide, Sickle Cell Anemia, Hemoglobin, Blood Flow, Pulmonary Hypertension, Echocardiogram

Summary: Sickle cell anemia is an autosomal recessive disorder and the most common genetic disease affecting African Americans. Approximately 0.15 percent of African Americans are homozygous for sickle cell disease, and 8 percent have sickle cell trait. Acute pain crisis, acute chest syndrome, and secondary pulmonary hypertension are common complications of sickle cell anemia. Mortality rates of sickle cell patients with pulmonary hypertension are significantly increased compared with patients without pulmonary hypertension. Recent studies report up to 40 percent mortality at 22 months after detection of elevated pulmonary artery pressures in sickle cell patients. Furthermore, pulmonary hypertension is thought to occur in up to 30 percent of clinic patients with sickle cell anemia. This study is designed to prospectively determine the prevalence and prognosis of secondary pulmonary hypertension in adult patients with sickle cell anemia using serial cardiac echocardiograms.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001175-01

LBC: CCM

Title: Delivery of NO by Hemoglobin to Improve Blood Flow in Sickle Cell Disease

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Mark T. Gladwin, M.D.

Supervisor of Record: Frederick P. Ognibene, M.D.

Collaborator, Lab: Robert L. Danner, M.D. (CCM, CC)

Collaborators, NIH: Richard O. Cannon, M.D. (CB, NHLBI)
James S. Nichols (CACPS, CC)
Alan N. Schechter, M.D. (LCB, NIDDK)

Total Staff Years: .5

Human Research: Human subject research

Keywords: Nitric Oxide, Sickle Cell Anemia, Hemoglobin, Blood Flow

Summary: Sickle cell anemia is an autosomal recessive disorder and the most common genetic disease affecting African Americans. Approximately 0.15 percent of African Americans are homozygous for sickle cell disease, and 8 percent have sickle cell trait. Acute pain crisis and acute chest syndrome are common complications of sickle cell anemia. Inhaled nitric oxide (NO) has been proposed as a possible therapy for the acute chest syndrome. Anecdotally, NO has been described to rapidly improve the hypoxemia and the clinical course of the acute chest syndrome. Furthermore, a number of recent studies have suggested that NO may have a favorable impact on sickle red cells at the molecular level and could improve the abnormal microvascular perfusion that is characteristic of sickle cell anemia. This clinical trial is designed to test the hypotheses that (1) individuals with sickle cell anemia have endothelial dysfunction with reduced local synthesis and release of NO, which may reduce regional perfusion at rest and impair the vasodilator response to stress, and (2) during NO inhalation, delivery of NO bound to hemoglobin will be enhanced and will improve these abnormalities in regional vascular perfusion. Studies will be performed on untreated sickle cell anemia patients and on patients managed with chronic hydroxyurea therapy. Demonstration of improved regional perfusion with NO therapy could have significant implications for patient management during acute pain crisis and the acute chest syndrome.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001176-01

LBC: CCM

Title: Effects of Endothelium-derived Versus Hemoglobin-transported Nitric Oxide

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Mark T. Gladwin, M.D.

Supervisor of Record: Henry Masur, M.D.

Total Staff Years: .25

Human Research: Human subject research

Keywords: Nitric Oxide, Sickle Cell Anemia, Hemoglobin, Blood Flow

Summary: Nitric oxide (NO) is a soluble gas that is continuously synthesized by the endothelium, and contributes importantly to vasodilator tone of the coronary and systemic circulations by activating guanylyl cyclase in vascular smooth muscle, causing relaxation. Although regional synthesis of NO by the endothelium contributes to local vasodilator tone, Stamler and coworkers have proposed that regional vascular tone may also be regulated by NO transported from the lungs by hemoglobin as a consequence of enhanced binding of NO to reactive thiols of oxygenated hemoglobin. This study is designed to determine the contribution of hemoglobin-transported NO to forearm microvascular dilator tone in healthy subjects, at rest and during regional hypoxia associated with forearm exercise stress. Measurements will be made before and after regional blockade of endothelial NO synthesis. Findings in this study may be relevant to understanding the physiological contribution and therapeutic potential of hemoglobin-transported NO in the regulation of vasodilator tone in diseases and conditions associated with regional endothelial dysfunction and reduced endothelial NO bioactivity (e.g., hypertension, diabetes mellitus, hypercholesterolemia, cigarette smoking, and estrogen deficiency).

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001177-01

LBC: CCM

Title: Influence of Systemic Inflammation on the Effects of Recombinant Granulocyte Colony-stimulating Factor in Sepsis

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Peter Q. Eichacker, M.D.

Supervisor of Record: Henry Masur, M.D.

Collaborator, Lab: Xizhong Cui (CCM, CC)

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Summary: Preclinical and clinical studies showed that treatment with recombinant granulocyte colony-stimulating factor (G-CSF) could improve host defense and reduce the risk of infection and sepsis in neutropenic patients. Preclinical studies suggested that treatment with G-CSF might also be beneficial during sepsis in the immunocompetent host. Despite these findings, G-CSF in early studies has not been beneficial in nonneutropenic patients with sepsis. Possibly consistent with this clinical experience, we found in a series of canine studies that G-CSF treatment had variable effects with *E. coli* bacteria challenges at differing sites and in differing dosages. To better determine the influence of these two factors on the effects of G-CSF; we developed a rat model of sepsis incorporating a multifactorial study design that permits simultaneous assessment of multiple variables. In these studies, with intravenous *E. coli* challenge, G-CSF increased lethality at all bacterial dosages. However, with intrabronchial and intraperitoneal challenge, G-CSF improved survival with low- and high-bacterial dosages but appeared harmful with an intermediate dosage. Laboratory investigations suggest that the variable effects of G-CSF on outcome in this study are related in part to its ability to increase circulating neutrophil numbers and the clearance of microbial and host inflammatory mediators. High levels of circulating bacteria, endotoxin, and TNF were associated with inhibition of both of these G-CSF effects. Clinically, this study suggests that G-CSF may have its greatest beneficial effect in patients with extravascular infection and limited bacteremia. Further studies are being designed to determine whether the harmful effects of G-CSF with intravascular challenge are related specifically to mediators associated with systemic inflammation, such as TNF.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001178-01

LBC: CCM

Title: Site, Severity, and Infection Type Influence on Superoxide Dismutase Effects in Sepsis

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Peter Q. Eichacker, M.D.

Supervisor of Record: Henry Masur, M.D.

Collaborator, Lab: Xizhong Cui (CCM, CC)

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Summary: Clinical experience with anti-inflammatory agents in patients with sepsis has been disappointing to date. We have found that several factors—such as the site, type, and severity of infection—have important influences on many of these agents. Developing new agents that are impacted minimally by such factors will increase the usefulness of this therapeutic approach. Oxidant injury is an important downstream event in the pathogenesis of sepsis. Targeting oxidants involved in this injury may be a more generally useful goal in sepsis. The enzyme superoxide dismutase (SOD) is an important regulator of oxidant injury. However, attempts to use recombinant SOD to limit inflammatory injury have been difficult because of its size and poor bioavailability. We are therefore now studying the influence of the factors listed above on the effects of a synthetic SOD mimetic agent, which is small and has good bioavailability, in a rat model of sepsis.

HOSPITAL EPIDEMIOLOGY SERVICE

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL080001-04

LBC: EPID

Title: Blood Exposures among Workers at the Clinical Center and Four Hospitals in Japan

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: David K. Henderson, M.D.

Total Staff Years: .2

Human Research: Human subject research

Keywords: Blood Exposures, Bloodborne Pathogens

Summary: This study is designed (1) to evaluate and compare nurses' knowledge of the epidemiology, pathogenesis, occupational risks, and appropriate prevention strategies for managing patients infected with bloodborne pathogens in the health care setting in four university hospitals in Japan and at the Clinical Center of the National Institutes of Health; (2) to compare self-reported levels of compliance with existing infection control recommendations designed to limit risk for exposure to bloodborne pathogens in all eight institutions; (3) to compare self-reported frequencies of cutaneous exposures to blood at the five hospitals in the study; and (4) to evaluate the effect of educational intervention on nurses' perceived compliance with recommendations and on the frequency of self-reported exposures to blood. Approximately 2,000 Japanese and 500 Clinical Center nurses have completed the initial survey. Preliminary data analysis demonstrates a substantial difference in knowledge about bloodborne pathogens and a corresponding difference in frequency of blood exposures among the hospitals studied. An educational intervention has been designed, and the first phase has been implemented. Nurses from Japan and the United States were resurveyed in early 2001, and the preliminary results appear to demonstrate substantial improvement in isolation technique and decreases in occupational exposures to blood.

DIAGNOSTIC RADIOLOGY DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL000077-04

LBC: DRD

Title: Treatment of Acute Deep Vein Thrombosis of the Lower Extremity with Intraclot, Pulse-sprayed Recombinant Tissue Plasmigen Activator

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Richard Chang, M.D.

Supervisor of Record: King Li, M.D.

Collaborators, Lab: Richard O. Cannon, M.D. (DDR, CC)
Clara Chen (DDR, CC)
Thomas Shawker, M.D. (DDR, CC)
Bradford J. Wood, M.D. (DDR, CC)

Collaborator, NIH: McDonald Horne, III (HEME, CC)

Total Staff Years: 3

Human Research: Human subject research

Keywords: Deep Vein Thrombosis, Thrombosis

Summary: The objective of the study is to evaluate recombinant tissue plasminogen activator (rtPA) for treatment of acute deep vein thrombosis of the lower extremity. The study is designed to evaluate the efficacy, safety, and cost of this form of treatment for restoration of venous function in the lower extremity. Eight patients have been treated. All except one patient had significant improvement. Only two patients have had evidence of small pulmonary emboli during treatment. These were detected on ventilation perfusion lung scans obtained for all patients accepted into the protocol. None of these patients were clinically symptomatic. One patient developed a non-life-threatening biceps hematoma, probably induced by automatic blood pressure monitoring during the rtPA treatment. No other complications have occurred. Preliminary results have been submitted for publication in a case report for *JAMA*, and in an article submitted to the *American Journal of Medicine.*

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000080-03

LBC: DRD

Title: Sonographic Evaluation of the Effects of Raloxifene on the Uterus and Ovaries in Premenopausal Patients

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ahalya Premkumar

Supervisor of Record: King Li, M.D.

Collaborators, Lab: Nilo Avila, M.D. (DDR, CC)
Georgia J. Cusack (DDR, CC)
Jennifer T. Goldstein (DDR, CC)
Diane A. Johnson (DDR, CC)
Marianne H. Noone (DDR, CC)
Anna Parsons, M.D. (DDR, CC)
David J. Venzon, Ph.D. (DDR, CC)
Joanne N. Zujewski (DDR, CC)

Total Staff Years: 2.9

Human Research: Human subject research

Keywords: Raloxifene, Postmenopausal, Breast Cancer

Summary: This protocol was developed as a companion protocol to #98-CC-0123. It allows us to study the reproductive effects of raloxifene in premenopausal women by transvaginal color Doppler sonography and sonohysterography with correlation to steroid hormones. Raloxifene is a selective estrogen-modulating agent that is being evaluated as a potential chemopreventive agent in patients at high risk for breast cancer. The safety and efficacy of raloxifene are being evaluated under protocol #98-CC-0123. Little data are available regarding the gynecological effects of raloxifene in premenopausal women. The purpose of our study is to study both the short- and long-term effects of raloxifene on ovulation frequency, endometrial development, and cyclic function in general. The study started enrolling patients in January 1999. There are 25 patients enrolled currently. No complications have been encountered so far, and we plan to continue this study.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000082-03

LBC: DRD

Title: Contrast-enhanced Magnetic Resonance Angiography in the Diagnosis of Atherosclerotic Disease: A Pilot Study

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Peter Choyke, M.D.

Supervisor of Record: King Li, M.D.

Collaborators, Lab:
Vincent B. Ho, M.D. (DDR, CC)
Ronald M. Summers, M.D., Ph.D. (DDR, CC)
Yantian J. Zhang (DDR, CC)
Peter J. Yim, Ph.D. (DDR, CC)

Collaborators, Extramural:
Bradley Dick, M.D., Chief, Interventional Radiology, Suburban Hospital
Thomas Foo, Ph.D., General Electric Medical Systems
Mario Gomes, M.D., Department of Surgery, Georgetown University
Conor Lundergan, M.D., Department of Cardiology
George Washington Medical Center
Azita Moalemi, M.D., Cardiology, Mt. Vernon Cardiology
Behram Pastakia, M.D., Radiology, Washington VA Medical Center

Total Staff Years: 2.6

Human Research: Human subject research

Keywords: Magnetic Resonance Angiography, Atherosclerotic Disease

Summary: The purpose of this protocol is to test technical improvements in magnetic resonance angiography (MRA). In order to improve our ability to respond to requests for MRA, we have initiated this protocol to recruit patients from the metropolitan Washington area who have peripheral vascular disease. To date, we have recruited 26 individuals with atherosclerosis. We have been able to investigate new methods of imaging these diseased vessels. For instance, we are evaluating time resolved (8-second) carotid MRAs using correlation imaging. We are evaluating high-resolution imaging of the calf vessels in order to improve the resolution of these small vessels. The results are as yet preliminary but very promising. There have been no complications. We plan to continue to recruit patients to this protocol over the coming year and anticipate large enough accruals to develop correlation imaging for the carotids and high resolution calf vessel imaging.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000084-03

LBC: DRD

Title: Diagnostic Efficacy of Virtual Bronchoscopy

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ronald M. Summers, M.D., Ph.D.

Collaborators, NIH: Steven Finkelstein, M.D. (NCI)
David Schrump, M.D. (TOS, NCI)
Michael C. Sneller, M.D. (IDS, NIAID)

Total Staff Years: 2

Human Research: Human subject research: Minors

Keywords: Virtual Bronchoscopy

Summary: This project is a test of the efficacy of a new diagnostic method for imaging the airways known as virtual bronchoscopy. Virtual bronchoscopy is performed by acquiring thin-section computer tomography (CT) images of the chest. These images are used to generate a three-dimensional (3D) model of the tracheal and bronchial walls on a graphics workstation. The model can be manipulated to allow the viewer to "fly through" the tracheobronchial tree, providing views similar to those obtained using bronchoscopy. The technique produces a display of the human bronchial system in a readily understood format. Moreover, it allows investigation of post-stenotic portions of the bronchial tree that are beyond the reach of fiberoptic bronchoscopy. Further, virtual bronchoscopy may be used to guide interventional procedures. The patients studied in this protocol will be those having inflammatory, infectious, or neoplastic pulmonary processes, who would have had chest CT for clinical reasons. These patients will be recruited from current NIH protocols. The study design consists of scanning of the thorax using thin-section helical CT, followed by 3D surface rendering of the airways and transfer of the digital data to videotape. In one of the four parts of the protocol, the virtual bronchoscopy will be compared with results from fiberoptic bronchoscopy in a blinded study. In a second part of the protocol, the virtual bronchoscopy will be used to perform a descriptive analysis of cavity lung lesions. In the third part, the utility of virtual bronchoscopy in diagnosis of neoplastic lesions of the chest will be studied. In the fourth part, certain technical problems in the virtual bronchoscopy procedure will be investigated. The patients will have only fiberoptic bronchoscopy for clinically indicated purposes. We anticipate that virtual bronchoscopy will be diagnostically efficacious for disorders that produce a morphologic alteration in bronchial anatomy. There have been no complications. Virtual bronchoscopy has been shown to be useful for detecting stenoses. We now have access to a CT scanner with higher Z-axis resolution and are investigating its efficacy for virtual bronchoscopy.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000085-03

LBC: DRD

Title: Normal Volunteer Scanning on Magnetic Resonance

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Peter Choyke, M.D.

Supervisor of Record: King Li, M.D.

Collaborators, Lab: John Butman, M.D., Ph.D. (DDR, CC)
Vincent B. Ho, M.D. (DDR, CC)
Nicholas Patronas, M.D. (DDR, CC)
Frances T. Sheehan (DDR, CC)
Ronald M. Summers, M.D., Ph.D. (DDR, CC)
Yantian J. Zhang (DDR, CC)

Total Staff Years: 3

Human Research: Human subject research

Keywords: Magnetic Resonance Imaging, MRI

Summary: The purpose of this protocol is to develop novel methods of performing magnetic resonance imaging (MRI) evaluations so that these methods can be transferred to the clinical environment. Normal volunteers are recruited to optimize imaging techniques, and the protocol has been very successful in recruiting normal volunteers. Among the accomplishments of this protocol over the last year are optimizing contrast administration rates during magnetic resonance angiograms (MRAs), automatic table motion techniques for peripheral run-off magnetic resonance angiography, phase contrast angiography, motion tracking for knee and patella movement, functional MRI of the brain, gated MRI to image the soft palate, and stroke protocols. We have made substantial gains in technical development in all of these areas, and there have been no complications. We have developed protocols to be used in conjunction with Suburban Hospital in MRA, stroke, and cardiac imaging and will continue this study.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000086-03

LBC: DRD

Title: Examination of the Hemodynamics of the Portal Venous System in Normal Volunteers Using Magnetic Resonance Imaging

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Elizabeth C. Jones

Supervisor of Record: King Li, M.D.

Collaborator, Lab: William F. Pritchard, M.D., Ph.D. (DDR, CC)

Collaborators, NIH: Robert Jensen, M.D. (DDB, NIDDK)
Robert J. Lutz, Ph.D. (DBEPS, OD)
Monica C. Skarulis, M.D. (DIR, NIDDK)

Total Staff Years: 1.8

Human Research: Human subject research

Keywords: Portal Venous System, Magnetic Resonance Imaging, Blood Flow Patterns

Summary: There are three components of the study: (1) technical development to define the imaging protocol, (2) conducting the observational study of blood flow patterns in study subjects, and (3) defining the anatomy and flow parameters for construction of flow models. The development of the principal imaging protocol is complete; the portal anatomy and flow patterns can be reproducibly imaged. During the period of technical development, we have observed evidence of the phenomenon of blood streaming, which is the central hypothesis of our study. The observational study of subjects after fasting and after a meal started in July 1999. Twenty-two patients have volunteered to date. Preliminary anatomic and flow data have been collected for model construction; a preliminary model has been constructed. The magnetic resonance examinations of the patients enrolled in the pivotal study are completed. Suitable software for data analysis has become available, and data analysis is under way.

LBC: DRD

Title: Comparison of Contrast-enhanced Magnetic Resonance Angiography and Conventional Angiography in Diagnosis of Atherosclerotic Disease: A Pilot Study

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Peter Choyke, M.D.

Supervisor of Record: King Li, M.D.

Collaborators, Lab: Andrew E. Arai, M.D. (DDR, CC)
Bradley W. Dick (DDR, CC)
Vincent B. Ho, M.D. (DDR, CC)
Hani Marcos, M.D. (DDR, CC)
Wayne Olan, M.D. (DDR, CC)
Yantian J. Zhang (DDR, CC)

Total Staff Years: 1.1

Human Research: Human subject research

Keywords: Magnetic Resonance Angiography, Angiography

Summary: This study will evaluate ways to improve magnetic resonance angiography (MRA) for diagnosing atherosclerosis. Patients with atherosclerosis who have had conventional angiography at Suburban Hospital in Bethesda, Maryland, will be considered for this study. Those enrolled will have an MRA scan at Suburban Hospital within 72 hours of their conventional angiogram. This protocol enables state-of-the-art technology developed in the Diagnostic Radiology Department, Clinical Center to be applied to patients with atherosclerotic disease with angiographic correlation. We are currently investigating improved methods of k-space ordering to allow rapid scanning of the run-off vessels while obtaining high resolution images of the vessels. Additional investigations will include real-time MR “fluoroscopy” to provide better timing of the MR during intravenous gadolinium chelate administration. Improvements in the MRA “package” may allow substitution of MRA for conventional catheter angiography in the near future.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000088-02

LBC: DRD

Title: Use of "Interoperative" Quick Parathyroid Hormone Assay during Parathyroid Venous Sampling

Dates: from 10/01/2000 to 08/08/2001

Principal Investigator: Richard Chang, M.D.

Supervisor of Record: King Li, M.D.

Collaborators, Lab: Alan T. Remaley, M.D., Ph.D. (DDR, CC)
Monica C. Skarulis, M.D. (DDR, CC)
Allen M. Spiegel (DDR, CC)

Total Staff Years: 1.8

Human Research: Human subject research

Keywords: Parathyroid Venous Sampling

Summary: Parathyroid venous sampling remains the most precise localization technique for patients with failed parathyroid surgery, but it requires considerable experience on the part of the angiographer as well as prolonged catheter manipulation to sample the multiple small veins draining the neck and mediastinum. Knowledge of the presence of parathyroid hormone (PTH) gradients during the procedure would enable the angiographer to focus on a specific area, perhaps obtaining more detailed samples, and to shorten the procedure when significant gradients occur in the early samples. This protocol will not increase the accuracy of sampling but will shorten the procedure, reduce radiation exposure to the patient and to the interventional radiologist, and allow more precise localization by more focused sampling. The success of another protocol (94-DK-0195) has decreased the number of patients who need parathyroid venous sampling, so our original estimates of the frequency of the study are no longer valid. Currently, it would be difficult to assemble enough patients to complete this study in a timely fashion. Parathyroid venous sampling has proven effective in the few OR patients tested, and we no longer consider it to be investigational. It is now used in routine patient care when needed. No new subjects were enrolled this year. We have terminated this protocol.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000089-01

LBC: DRD

Title: Assessment of RAS and Renovascular Hypertension by Contrast-enhanced Magnetic Resonance Imaging

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Peter Choyke, M.D.

Supervisor of Record: King Li, M.D.

Collaborators, Lab: George R. Altizer (DDR, CC)
Clara Chen (DDR, CC)
Vincent B. Ho, M.D. (DDR, CC)
Jeffrey B. Kopp, M.D. (DDR, CC)
Hani Marcos, M.D. (DDR, CC)
Lalith Talagala, Ph.D. (DDR, CC)
Christopher Wilcox (DDR, CC)

Collaborator, NIH: Hugh L. Preas, II, M.D. (ANES, CC)

Total Staff Years: 1

Human Research: Human subject research

Keywords: Assessment of RAS and Renovascular Hypertension by Contrast MRI: Pilot

Summary: The purpose of this protocol is to determine whether magnetic resonance angiography (MRA) and captopril magnetic resonance (MR) renography can provide comprehensive evaluation of patients at risk for renovascular hypertension. Although renovascular hypertension (renal artery stenosis causing high blood pressure) is unusual, it nonetheless represents a correctable form of hypertension. The current methods of evaluating patients for renovascular hypertension are cumbersome and include Doppler sonography, captopril renography, MRA, and angiography. The purpose of this protocol is to test the current gold standards—captopril renography and angiography—against a combination of MRA and captopril MR renography. For this study, the at-risk patient undergoes a conventional captopril nuclear medicine renogram followed by an MR renogram and MRA. The patient also breathes an oxygen-rich gas, known as carbogen, which is used to test for renal ischemia. To date, we have accrued six volunteers and ten patients to this protocol. We anticipate continued patient accrual to this protocol over the coming year.

LABORATORY OF DIAGNOSTIC RADIOLOGY RESEARCH

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL008038-02

LBC: LDRR

Title: Magnetic Resonance Perfusion Imaging in Hypercapnia: Development of Technical Protocols

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Frank, M.D., M.S.

Collaborators, Lab: Bobbi K. Lewis (LDRR, CC)
Keith St. Lawrence, Ph.D. (LDRR, CC)

Collaborators, NIH: Alan Charles McLaughlin, Ph.D. (CBDB, NIMH)
Frank Ye, M.S. (DIRP, NIMH)

Total Staff Years: 2

Human Research: Human subject research

Keywords: Functional MRI, Carbogen, Cerebral Perfusion

Summary: Advances in magnetic resonance (MR) perfusion imaging have provided clinical researchers with the opportunity to measure quantitative regional increases in cerebral blood flow. The purpose of this study is to acquire the technical experience required to perform MR perfusion imaging studies of the hypercapnic cerebral blood flow response. Cerebral blood flow will be increased by inhalation of carbogen (an air mixture containing 5 percent CO2) or intravenous injection of the carbonic anhydrase inhibitor acetazolamide. The technical experience obtained in this study will be used to design a study of the pharmacological and physiological mechanisms underlying cerebral blood flow increases during hypercapnia.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090001-09

LBC: LDRR

Title: Magnetic Resonance Imaging in Multiple Sclerosis

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Frank, M.D., M.S.

Collaborators, Lab: Craig N. Bash, M.D. (LDRR, CC)
Thomas R. Howard (LDRR, CC)
Nancy Richert, M.D., Ph.D. (LDRR, CC)

Collaborators, NIH: Roland M. Martin, M.D. (U, NINDS)
Henry F. McFarland, M.D. (NIB, NINDS)

Total Staff Years: 4

Human Research: Human subject research

Keywords: Magnetic Resonance Imaging, Multiple Sclerosis

Summary: The focus of this project is the use of magnetic resonance imaging (MRI) to understand the pathophysiology of multiple sclerosis (MS) and to determine whether disease activity is altered by various immunomodulatory treatments such as Anti-Tac antibodies or Roliprom, a phosphodiesterase 4 inhibitor, and to monitor the natural history of MS. Magnetization transfer (MT) imaging, which is sensitive to the amount of bound and free water in the white matter and indirectly reflects myelination, was also used to evaluate these patients. MT region of interest (ROI) analysis of individual enhancing or nonenhancing lesions reveals that there is no difference in the pattern of MS lesion recovery either when a lesion develops during the natural history of the disease or when receiving INFB-1b. However, there does appear to be a faster improvement in the MTR recovery toward baseline when enhancing lesions occur in association with a clinical exacerbation requiring treatment with intravenous steroids. These results would indicate that closure of the blood-brain barrier with steroids is associated with a decrease in the ratio of free to bound water within an MS lesion that is detected as a change in MTR. MTR studies performed of MS lesions in patients receiving rhIGF-1 suggest that a similar recovery pattern is observed with steroids, which would be consistent with the proposed anti-inflammatory effects of this agent. In 2000, the open-labeled baseline vs. treatment trial is evaluating altered peptide ligand (APL) as a treatment for relapsing-remitting MS patients closed because of significant severe adverse events. APL is thought to interfere with binding of T-cells to antigen-presenting cells and to induce tolerance in the MS patients. APL appeared to alter the T-cells to pro-inflammatory phenotype, thus possibly stimulating MS disease progression as documented on MRI. Further immunologic-imaging evaluations of the patients with unresponsive MS to conventional therapy is ongoing using Anti-Tac antibodies directed against T-cells, and in five patients the combination of interferon and Anti-Tac antibodies is well tolerated, with a decrease in enhancing lesions. A phase II trial is under way evaluating the new oral agent, Roliprom, for the treatment of relapsing-remitting MS patients, using suppression of frequency of enhancing lesions as an outcome measure.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090003-07

LBC: LDRR

Title: Functional and Metabolic Imaging in the Brain

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Frank, M.D., M.S.

Collaborators, Lab: Bobbi K. Lewis (LDRR, CC)
Keith St. Lawrence, Ph.D. (LDRR, CC)

Collaborators, NIH: Alan Charles McLaughlin, Ph.D. (CBDB, NIMH)
Frank Ye, M.S. (DIRP, NIMH)

Total Staff Years: 3.25

Human Research: Human subject research

Keywords: Imaging of the Brain, MRI, Magnetic Resonance Imaging

Summary: Functional and metabolic magnetic resonance imaging (MRI) techniques have been rapidly evolving and have tremendous potential for clinical brain disorder research. Clinical activation MRI studies are performed at 1.5 and 3.0 Tesla, using blood oxygenation level dependent (BOLD) contrast method and arterial spin tagging (AST) techniques. Reproducible alterations cerebral blood flow (CBF) have been performed by having healthy controls inhale carbogen at 6 percent carbon dioxide with an approximate increase of 20 to 30 percent in CBF. These preliminary studies allowed us to perform pharmacological challenges using a cyclo-oxygenase inhibitor (COX) 1, indomethicin, and have demonstrated an almost complete suppression of the alteration of CBF to 6 percent carbon dioxide in subjects at rest. Plans are to evaluate the changes in CBF to other COX inhibitors to determine the response of the cerebral endothelial cells. In addition, we are planning to perform these studies during sensorimotor task activation in order to determine if COX-1 and COX-2 drugs suppress the CBF response with stimulation. Future work will focus on improving the AST pulse sequences with background suppression to provide coverage over the whole head and also move the techniques to higher field strengths.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090004-08

LBC: LDRR

Title: Development and Evaluation of Magnetic Resonance Contrast Agents

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Joseph A. Frank, M.D., M.S.

Collaborators, Lab: Jeff W.M. Bulte, Ph.D. (LDRR, CC)
E. Kay Jordan, D.V.M. (LDRR, CC)
Bobbi K. Lewis (LDRR, CC)
Holly Zwickie, B.S. (LDRR, CC)

Collaborators, NIH: Martin W. Brechbiel (ROB, NCI)
L. Henry Bryant, Ph.D. (CC)

Collaborator, Extramural: Trevor Douglas, Ph.D., Chemistry
Temple University

Total Staff Years: 2.25

Human Research: Neither human cells nor tissues

Keywords: Magnetic Resonance Contrast Agents

Summary: STAR BURST dendrimers (D) and ultra small iron oxide particles (USPIO) were developed as cellular tags in molecular imaging. A series of high generation (G) dendrimers (G = 5, 7, 9, 10) were conjugated to DOTA, and gadolinium (III) ion was added to the 1/T1 and 1/T2 NMR dispersion profile for generations 5 through 10 dendrimer DOTA complexes. There is an increase in the proton relaxation enhancement effect (PRE) from G 5 through G 7, which levels off at generations 9 and 10. Biodistribution studies in rodents using radiolabeled gadolinium 153 chelated to the DOTA-dendrimer complex reveal dose-dependent effect for blood half-life and tissue distribution. Cellular labeling was accomplished by incubating G9DOTA-Gd with cells in culture with magnetic resonance imaging (MRI) of the cells, along with fluoresces studies indicating that G9DOTA-Gd was in the cytoplasm of cells. Long-term stability and cell viability studies are being planned in order to determine which generation of DOTA-Gd should be used as the basis for specific labeling to target receptors or for drug delivery. Magnetodendrimers (MD) are iron oxide particles coated with dendrimers. MD is a T2-star-shortening susceptibility contrast agent and is more efficient at shortening the T2-relaxation times of solution compared with other iron-oxide-based agents. The MD can also be used as a molecular label, as the dendrimers are used as transfection agents, and, therefore, the MD can easily be incorporated into the cytoplasm of various cell cultures, including malignancy and stem cells, by simple incubation. MD has been used to magnetically label adult neural stem cells (NSC) and mesenchymal stem cells (MSC). Both NSC and MSC incorporated the MD and were clearly visible on MR imaging at 1.5 Tesla at a level of 10e6 cells. MD-labeled NSC and MSC differentiated normally along appropriate cell lines. In addition, MD-labeled stem cells were still viable and dividing 10 days after labeling. MRI demonstrated that mouse embryonic stem cells labeled with MD were visualized when transplanted into a spinal cord crush injury model.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090005-06

LBC:	LDRR
Title:	Magnetic Resonance Imaging in Experimental Allergic Encephalomyelitis and Remyelination
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Joseph A. Frank, M.D., M.S.
Collaborators, Lab:	Jeff W.M. Bulte, Ph.D. (LDRR, CC) E. Kay Jordan, D.V.M. (LDRR, CC) Bobbi K. Lewis (LDRR, CC) Holly Zwickie, B.S. (LDRR, CC)
Collaborator, NIH:	Richard C. Saunders, Ph.D. (LN, NIMH)
Collaborators, Extramural:	Ian Duncan, D.V.M., Ph.D., Veterinary Medicine, University of Wisconsin John McDonald, M.D., Neurosurgery, Barnes Hospital
Total Staff Years:	3
Human Research:	Neither human cells nor tissues
Keywords:	Magnetic Resonance Imaging, Allergic Encephalomyelitis, Remyelination

Summary: Magnetic resonance (MR) imaging scans were performed in myelin-deficient animals in which magnetically labeled progenitor oligodendrocytes were implanted into the spinal cord. Three-dimensional MR microscopy (MRM) was performed *ex vivo* on the spinal cords with 78 micron isotropic resolution at 4.7 Tesla. MR microscopy showed extensive migration (up to 8.4 mm) of magnetically labeled grafted cells, particularly in the area of the dorsal column. MR images were correlated with histopathologic staining for iron, myelin, astrocytes, and microglia. Both the Prussian-blue and myelin staining closely matched the area of contrast enhancement seen on the MR images. Magnetically labeled precursor oligodendrocytes were implanted in the brains of dysmyelinated rats and cell proliferation and migration were noted at clinically relevant MRI 1.5 Tesla units. The labeled cells migrated along the surface of the lateral ventricle and into the olfactory lobe of the rat brain. These cells penetrated into the white matter and myelinated axons, as evident on Prussian-blue and myelin stains. Mouse embryonic stem cells (ESC) were labeled with magnetodendrimer and transplanted into a spinal cord crush injury model in collaboration with Dr. John McDonald. Magnetically tagged, labeled cells migrated into the area of crush injury and ESCs were noted to differentiate into neuronal elements on immunohistochemical stains in rats that were euthanized by day 14 post-injury. MRI images at 4.7 Tesla were able to demonstrate the extent of migration along the damaged spinal cord. Further studies are planned to optimize the time and number of magnetically labeled cells needed for transplantation in this spinal cord crush injury model. Serial MRI studies performed in the marmoset model experimental autoimmune encephalomyelitis (EAE) are used for preclinical evaluation of new therapies for multiple sclerosis. The serial MRI studies have demonstrated week-to-week changes in the number and distribution of EAE lesions. MRI stereotactic-directed biopsy techniques have been perfected that will allow us to biopsy EAE lesions for histopathologic evaluation of the cellular microenvironment of the EAE lesions. Studies are planned to use this model as a basis for stem cell transplantation to determine if these cells will stimulate remyelination in EAE lesions.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090006-09

LBC: LDRR

Title: Multimodality Radiological Image Processing System

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: R.L. Levin, D.Sc.

Total Staff Years: 1

Human Research: Neither human cells nor tissues

Keywords: Multimodality Radiological Image Processing System, Radiological Image

Summary: During this year, data from the MRIPS Archive and Retrieval System (MARS) is being migrated over to the Clinical Center's new Picture Archiving and Communication System. A new version of MEDx 3.4.1 was released this year. Enhancements to MEDx include a DICOM positron emission tomography reader and a new DICOM image manager. The perfusion module has also been improved and now allows users to manually specify arterial pixels.

NUCLEAR MEDICINE DEPARTMENT

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LBC: NMIP

Title: Imaging Organ Function in Small Animals

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Michael Green

Collaborators, Lab: Injae Lee, Ph.D. (DNM, CC)
Jurgen Seidel, Ph.D. (DNM, CC)
Juan Jose Vaquero, Ph.D. (DNM, CC)

Collaborators, NIH: Calvin A. Johnson, Ph.D. (IPRS, CIT)
James V. Sullivan (MIF, BEIP, OD)

Collaborator, Extramural: Fernando Barbosa, E.E.
Thomas Jefferson National Laboratory

Total Staff Years: 3.5

Human Research: Neither human cells nor tissues

Keywords: Small Animal PET, Small Animal SPECT, Small Animal Radionuclide Imaging

Summary: Investigations undertaken during the past several years identified optimal subsystem designs for a stationary ring, small animal positron emission tomography (PET) scanner with depth-of-interaction capability. During this reporting period, the first of two ATLAS (Advanced Technology Laboratory Animal Scanner) scanners based on these designs was mechanically and electronically completed. The second system, ATLAS II, was mechanically completed and all components were fabricated, including custom electronics boards and amplifier packages that include the 18 detector modules that surround the animal. The first and second systems differ from one another only in that the second system is based on Hamamatsu R7600 C-12 position-sensitive photomultiplier tubes (PSPMTs), whereas the first system is based on R7600 C-8 PSPMTs. This difference will allow ATLAS II (primarily a physics research machine) to be eventually upgraded to higher spatial resolution and, in the meantime, provide the Imaging Physics Laboratory with a research test bed for continuing experiments in high-resolution PET. ATLAS I is intended ultimately for routine use in the intramural research program to study organ function in small animals such as rats and genetically altered mice. Preliminary measurements made on ATLAS I indicate that the performance predictions made by computer simulation of the ATLAS design are accurate and that ATLAS meets virtually all of the design goals set for this system. Among these are the highest absolute central point source sensitivity of any system of comparable axial field-of-view and a resolution uniformity superior to any system of comparable ring diameter. During this reporting period, work commenced on a comprehensive software package and user interface for ATLAS, that includes a number of data acquisition protocols, a variety of local and remote image reconstruction options, and an extended image analysis and visualization package that includes multimodality image registration software. This latter capability will be exploited when the small-animal computer tomography (CT) scanner ordered this year is delivered and integrated into the study environment during the next reporting period. CT images obtained on each animal undergoing a PET study will be used to correct the PET image data for attenuation and to help identify structures labeled with the PET radiopharmaceutical.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000416-03

LBC: NMIP

Title: Image Analysis for Quantitative Assessment of Tumor Response to Therapy

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Stephen L. Bacharach, Ph.D.

Collaborators, Lab: Joann M. Carson (IPS, CC)
Senthil Kumar, M.D. (IPS, CC)
Philip M. Mansour (IPS, CC)

Collaborators, NIH: Jorge A. Carrasquillo, M.D. (DNM, CC)
Peter Choyke, M.D. (DDR, CC)
Steven K. Libutti (SMS, NCI)

Total Staff Years: 3

Human Research: Human subject research

Keywords: Tumor Response, Image Analysis, Quantitative Assessment

Summary: The Department of Nuclear Medicine, in conjunction with the National Cancer Institute and the Department of Radiology, performs clinical research in the use of imaging in oncology. In particular, they are studying the use of positron emission tomographic (PET) images, in conjunction with CT and MR images, to evaluate the effects of therapy on tumors. Several therapeutic agents are being studied, among them various anti-angiogenesis therapies. The PET scanners are used to measure glucose metabolism, blood flow, and blood volume in tumors over the course of therapy. CT scans are used to determine tumor morphology and MR imaging is used to determine both morphology and parameters related to tumor perfusion. This research is geared toward developing, implementing, and testing methods to better quantify the data obtained from the images and to determine if these methods are efficacious for the monitoring of tumor therapy. These methods involve determination of tumor morphology, as well as the optimal determination of functional parameters such as blood flow, metabolism, and blood volume. The overall goal is the development of a clinically useful method-ology for determining tumor response to therapy at an earlier phase of therapy than is currently possible. Such a methodology could permit optimal adjustment of the course of therapy while the therapy was still proceeding, potentially improving both tumor response and patient morbidity. Several areas of investigation are being pursued toward achieving this goal. Some of the principal ones are listed below. (1) Development of new reconstruction techniques that will improve noise properties of the image sets. This noise reduction will improve the ability of the physician to visually interpret the images, and improve the noise in quantitative parameters derived from the images. This work has been published in the *Journal of Nuclear Medicine* (Noise Reduction in Oncology FDG PET Images by Iterative Reconstruction: A Quantitative Assessment. *J Nucl. Med.* Sept. 2001;42:1316-1323). As a result of this work, all reconstructions in oncology are now performed with the new iterative reconstruction method. (2) Assessment of the physiologic models employed for blood-flow measurement, using O-15 water. Several models are being analyzed, especially with regard to their utility in producing functional flow images. In addition, the results of these PET flow models are being compared with similar data obtained from Gd-DTPA dynamic MR images. The variability and reproducibility of each of the methods is also being determined,

using replicate measurements. The first data from these studies has recently been published (Parametric Images of Blood Flow in Oncology PET Studies Using 15-O water. *J Nucl Med*. 2000; 41:1784-1792 and Measuring Tumor Blood Flow with H2O15: Practical Considerations. *Nuc. Med. Biol.* 2000;27:671-676). Further work will focus on better models to account for tumor heterogeneity. (3) Methods for making accurate, noninvasive measurement of the arterial input function are being assessed. These methods compare LV cavity and aorta derived arterial input functions with actual arterial sampling. Several schemes are being explored to correct for partial volume and spill in/out effects. The results of this work are being prepared for publication (the fellow performing the work has recently finished his fellowship). (4) Methods for using three-dimensional region-growing to more accurately assess tumor volume, metabolic volume, and perfused tumor volume are being explored. These methods will be employed to make objective assessments of the various physiologic parameters (e.g., FDG "uptake"), and ROC analysis will be used to determine which of these quantitative indices are best for detecting disease and to determine if quantitative measures are better than subjective visual assessment. (5) The results of the above four methodologies have recently been applied in practice to the first completed protocol (Thalidomide for Prostate Metastases). The clinical results of this analysis are being submitted for publication.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000600-08

LBC: NMRR

Title: Radiolabeled Monoclonal Antibody Imaging of Tumors and Positron Emission Tomography Oncology

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Jorge A. Carrasquillo, M.D.

Collaborators, Lab: Chang Hum Paik, Ph.D. (DNM, CC)
Luke S. Park (DNM, CC)
Karen J. Wong (DNM, CC)
Sarah Yu (DNM, CC)

Collaborators, NIH: Martin Brechbiel, Ph.D. (ROB, NCI)
Ira Pastan, M.D., Ph.D. (LMB, NCI)
Thomas A. Waldmann, M.D., Ph.D. (MB, NCI)

Total Staff Years: 4.8

Human Research: Human subject research

Keywords: Radiolabeled Monoclonal Antibody Imaging, Tumors, Positron Emission Tomography

Summary: These studies are designed to develop improved methods for detecting and treating malignancies. Our group performs preclinical evaluation of antibodies that appear to be promising after initial screening by various laboratories at the National Cancer Institute and develops these antibodies for clinical application. The clinical trials evaluating their pharmacokinetics and dosimetry are performed by our group. A collaborative radioimmunotherapy trial with Dr. Waldmann (Principal Investigator), in which we use humanized anti-tac monoclonal antibody, is ongoing. A collaborative radioimmunotherapy trial with bone marrow support is ongoing with NCI. Various protocols using (F-18) FDG in positron emission tomography and (O-15) water for tumor detection, followup, and blood-flow measurements are ongoing. We have begun preclinical studies evaluating pretargeting of antibodies for tumor therapy and have demonstrated therapeutic responses with Y-90 and Bi-213 in animal tumor models.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002001-08

LBC: NMRR

Title: Chemical Modifications of Antibodies for Tumor Targeting

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Chang Hum Paik, Ph.D.

Supervisor of Record: Jorge A. Carrasquillo, M.D.

Collaborators, Lab: Luke S. Park (DNM, CC)
Karen J. Wong (DNM, CC)

Collaborators, NIH: Martin Brechbiel, Ph.D. (ROB, NCI)
Ira Pastan, M.D., Ph.D. (LMB, NCI)
Thomas A. Waldmann, M.D., Ph.D. (MB, NCI)

Total Staff Years: 3

Human Research: Neither human cells nor tissues

Keywords: Tumor Targeting, Chemical Modifications

Summary: This project was developed and is directed by C.H. Paik, Ph.D. The research has centered on improving the tumor-targeting property of monoclonal antibodies and fragments by chemical modifications. For FY2000, we have investigated the use of a novel multi-step tumor targeting. Our strategy involves pretargeting tumors with monoclonal antibody (MoAb) peptide, followed by the injection of a radiolabeled second peptide. This approach decouples the antibody injection from the radiolabel injection, thereby offering the specificity of antibody binding to tumor antigens while eliminating problems associated with slow blood clearance of radiolabeled MoAb due to its large size. This project involves the synthesis of MoAb-peptide conjugates, clearing agents, and radiolabeled peptides. We have been optimizing the synthesis of these reagents to maintain the integrity of the immunoreactivity of MoAb and to allow dimerization formation of peptides. Our preliminary multistep tumor-targeting experiments using tumor-bearing nude mice showed high accumulation of a radio-labeled peptide in tumor tissues and low accumulation in nontumor tissues and blood, thereby providing high tumor-to-background radioactivity ratios.

LBC: NMRR

Title: Gene-Specific Radiotherapy

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Ronald D. Neumann, M.D.

Collaborators, Lab: Igor Panyutin, Ph.D. (DNM, CC)
Thomas A. Winters, Ph.D. (DNM, CC)
Irina Panyutin, M.D. (DNM, CC)
Elzbieta Pastwa, Ph.D. (DNM, CC)
Yelena Gaidamakova, Ph.D. (DNM, CC)

Total Staff Years: 6.5

Human Research: Neither human cells nor tissues

Keywords: Gene-Specific Radiotherapy

Summary: The goal of this project is the development of therapeutic radiopharmaceuticals based on targeting the decay of Auger-electron-emitting radioisotopes to specific sequences in DNA (genes) using triplex-forming oligonucleotides (TFOs) as delivery vehicles. In *in vitro* studies, we have demonstrated that TFOs are able to deliver Auger electron emitters to specific targets in cellular DNA in order to inactivate genes and/or kill the cells containing the target sequences. Decay of I-125 in TFOs results in strand breaks in both strands of the target DNA with an efficiency from 0.4 to 0.8 break/decay. Higher efficiency can be achieved with radionuclide multiple labeling. Breaks are confined to the triplex target sequence, and 90 percent of the sequence-specific breaks are located within 10 bp of the decay site. We showed that radiotoxicity of TFOs delivered into the cell nucleus as measured by clonogenic assay is 300 times less than that of DNA-incorporated I-125UdR. TFOs were designed to target the human MDR1 gene that is amplified in KB-VI cells in culture. The TFOs were labeled with I-125, and the targeting was detected by the presence of radioiodine-induced breaks. The breaks were found in DNA purified from I-125-TFO-treated isolated nuclei and digitonin-permeabelized cells. To increase the efficiency of targeting, a new generation of chemically modified oligonucleotides is being developed with increased *in vivo* stability, permitting one-step labeling with Auger electron emitters. We have developed a rapid procedure for incorporation of the short-life Auger electron emitters I-123 and I-111In-111 into ODNs and demonstrated that decay of these more clinically relevant radioisotopes produces DNA breaks with a yield comparable to that of I-125. We also have shown that the fine structure of DNA damage by decay of Auger electron emitter depends on local DNA conformation and that, by analyzing the DNA damage, one can obtain information on the structure of DNA in nucleoprotein complexes both *in vitro* and *in vivo*. Based on this principle, a new method of radioprobing DNA-protein complexes has been demonstrated in several model systems. In addition, studies have been initiated to investigate the mechanisms of Auger-electron-induced DNA strand break repair in human cells. We have developed efficient methods of producing and isolating specific forms (form I and form II) of damaged shuttle-vector plasmid DNA, using both oxidative agents and TFO-bound Auger-emitting radionuclides as damaging agents. A liposome delivery system has been developed for efficient delivery of damaged DNA into human cells in order to evaluate the *in vivo* repairability and mutagenicity of site-specific DNA double-strand breaks (DSBs) induced by I-125-labeled TFOs.

Using the methods described above, I-125-TFO-induced DNA DSBs were found to be very effective at inactivating a shuttle-vector-borne target reporter gene by mutagenic disruption. The mutation frequency for I-125-TFO-induced DSB was approximately 80 percent, and the mutation spectrum was dominated by multiple base deletions involving the targeted I-125 decay site. The I-125-TFO-induced DSB was also approximately 100 times more refractory to repair than oxidatively induced DSB, similar to those produced by ionizing radiation and reactive oxygen species (ROS) such as hydroxyl radicals. *In vitro* DSB repair assays have been developed to permit isolation of human proteins that are involved in DSB repair and to analyze DNA reaction products at the molecular level for comparison with DNA repaired *in vivo*. This assay employs plasmid DNA containing a DSB similar to that produced by ionizing radiation and other ROS. This DSB lesion more closely models naturally occurring DSB than DSB produced by other methods, such as restriction enzymes. In support of this assay, methods have been developed to produce and recover the large quantities of plasmid substrate DNA (linearized by bleomycin) necessary for chromatography and biochemistry procedures. The assay has been optimized for DSB rejoining using human HeLa cell extracts. Optimal conditions depend on the complexity of DSB introduced into the substrate DNA, with slight variations of pH and ionic strength being the variables. Standard reaction conditions have been established, and, under these conditions, the initial-repair-reaction rate for complex DSB produced by bleomycin is approximately twofold less than for the equivalent, but less chemically complex, restriction-enzyme-produced DSB. The goals of the studies outlined above are to identify the human repair pathways involved in Auger-emitter-induced DSB repair; assess the consequences of repairing these lesions; and examine methods by which these repair processes can be manipulated to augment the radiotherapeutic effects of TFOs labeled with Auger-electron-emitting radionuclides.

POSITRON EMISSION TOMOGRAPHY DEPARTMENT

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LBC: NPET

Title: Development of New Radiopharmaceuticals and New Paradigms in Positron Emission Tomography

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: William C. Eckelman, Ph.D.

Collaborators, Lab: Richard E. Carson (PETD, CC)
Peter Herscovitch, M.D. (PETD, CC)
Elaine M. Jagoda (PETD, CC)
Chih-Hao K. Kao (PETD, CC)
Dale O. Kiesewetter (PETD, CC)
Lixin Lang (PETD, CC)
Lawrence P. Szajek (PETD, CC)

Total Staff Years: 6.5

Human Research: Human subject research

Keywords: Radiopharmaceuticals, Positron Emission Tomography

Summary: We previously described the radiosynthesis and preliminary biodistribution of 3-(3-(3-[F-18]fluoropropyl)thio)-1,2,5,thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine ([F-18]FP-TZTP, a muscarinic M2 selective ligand. In collaboration with National Institute of Mental Health investigators, we recently began studies in normal human volunteers, with the eventual goal of studying patients with Alzheimer's disease. The initial analysis, based on data from six young control subjects, concentrated on the determination of the appropriate kinetic model for [F-18]FP-TZTP in humans. In plasma, parent compound represented 68 plus or minus 8, 41 plus or minus 9, and 14 plus or minus 4 percent of radioactivity at 20, 40, and 120 minutes, respectively. The plasma-free fraction (fp) was 5.9 plus or minus 1.2 percent. A model with one tissue compartment produced an excellent fit for the full 120 min of data, so that the additional parameters of a two-compartment model were unidentifiable. K1 values in gray matter regions were high—0.36 to 0.56 ml/min/ml—and showed excellent correlation with CBF. V values, representing total tissue binding, were very similar in cortical regions, basal ganglia, and thalamus but were significantly higher ($p < .01$) in amygdala. Unlike the results in the monkey, binding in the cerebellum was similar to that in the cerebral cortex. V values correlated with fp and normalization of V by fp reduced the coefficient of variation of V from 24 to 16 percent. The methodology and results from our analysis of [F-18]FP-TZTP data in young controls provide the basis for ongoing studies in elderly controls and patients with Alzheimer's disease. Another goal is to develop a series of F-18-labeled radiopharmaceuticals that are serotonin 5-HT-1A subtype specific and have a range of binding affinities. We evaluated [F-18]FCWAY in rhesus monkeys with PET. The goal of these studies was to further develop [F-18]FCWAY as a radioligand to measure 5-HT-1A receptors in humans. Studies included control experiments to develop an appropriate kinetic model and preblocking studies to demonstrate specific binding and to estimate the level of nonspecific binding. Separate experiments with injection of the labeled metabolites of [F-18]FCWAY were also performed to determine if significant brain accumulation occurred. Control [F-18]FCWAY studies were performed to assess the regional binding patterns. Like [C-11]WAY, [F-18]FCWAY showed

a binding pattern consistent with 5-HT-1A binding, that is, the highest binding in the frontal and medial temporal cortex, intermediate binding in other cortical regions, low binding in the thalamus and caudate nuclei, and minimal uptake in the cerebellum. By 60 to 90 minutes post-injection, the frontal-to-cerebellum binding ratio was a difference of 10:1. Preblocking studies with WAY 100635 (200 nmol/kg) administered 5 min before [F-18]FCWAY administration showed complete removal of specific binding, that is, highly uniform low binding levels in all regions. Longer-lived PET radionuclides (Br-76 and I-124) are under development, along with the technetium isotope Tc-94m. We also continue to produce At-211 for use in alpha particle therapy.

LABORATORY MEDICINE DEPARTMENT

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LBC: DLM

Title: Analytical Methodology: Development and Interpretive Application

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Nadja Rehak

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Stacey A. Cecco (CCS, CC)

Total Staff Years: .1

Human Research: Neither human cells nor tissues

Keywords: Analytical Methodology

Summary: Plasma is often the preferred specimen type when the goal of the laboratory is to reduce the turnaround time and clotting occurrences of the analytical systems. Recently, blood collection tube containing Li heparin as anticoagulant and inert gel material to separate the blood cells from plasma was introduced for routine laboratory use (Becton Dickinson Vacutainer Plus #7961). We evaluated this plasma separator tube (PST) with Hitachi 917 for direct sampling and storage effect on 27 commonly measured analytes. Blood was collected into PST and serum separator tubes (SST) (Becton Dickinson Vacutainer Plus #7983) from volunteers (n = 30), and for additional studies, into Li heparin anticoagulated tubes (PT) (Becton Dickinson Vacutainer Plus #7886). All tubes were centrifuged at the routine setting for the SST processing (RCF = 2190 xg, 5 min) within 30 minutes of collection. Samples were analyzed in batch mode from the primary tubes with Hitachi 917 fresh, and after storage for 8 hours at room temperature (rt) and up to 5 days at 4°C. Significance of the mean difference (mdiff) was based on the imprecision of the method. In comparison with fresh serum, fresh plasma collected in PST had significantly different (mdiff1 = plasma – serum) concentration of K (mdiff1 = –0.23 mmol/L), albumin (mdiff1 = 0.1 g/dL), total protein (mdiff1 = 0.13 g/dL) and activity of ALT (mdiff1 = –7 U/L), and LD (mdiff1=25 U/L). None of the measured analytes were affected by the storage of samples in PST and SST for 8 hours at rt. The effect of extended storage at 4°C (mdiff2 = stored – fresh) on plasma analytes of PST specimens was significant for bicarbonate (–5 mmol/L), K (0.9 mmol/L), Pi (–0.5 mg/dL), glucose (–7 mg/dL), and LD (39 U/L). In comparison, storage of serum in SST affected only LD activity (–10 U/L). The LD activity for fresh PST plasma was comparable to that for fresh PT plasma (mean: 180 and 174 U/L respectively). The extended storage of plasma in PST and PT at 4°C caused an increase in the activity of LD (PST: mdiff2 = 30 U/L, PT: mdiff2 = 24 U/L), indicating incomplete separation of platelets by the gel barrier in PST. However, the PST plasma LD isoenzymes were not affected by storage at 4°C. In addition, the increase in the PST plasma LD activity was not related to the centrifugation time (5, 8, and 10 minutes) or to the plasma platelet count. However, we observed that all centrifuged PSTs had varying amounts of erythrocytes imbedded in the gel and/or on top of the gel. This strongly suggests that the observed changes in LD and K were due to incomplete separation of plasma from red blood cells. In conclusion, we found PST to be acceptable for blood collection of heparinized plasma when appropriate changes in reference intervals for ALT, LD, and K are implemented. Furthermore, most of the common analytes are stable for up to 8 hour at ambient room temperature. However, the gel barrier does not provide satisfactory separation of plasma and cells and, therefore, the extended storage of plasma in primary tubes at 4°C is not advisable.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010010-26

LBC: DLM

Title: Magnesium Metabolism in Humans and Biological Systems

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Nadja Rehak

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Stacey A. Cecco (CCS, CC)

Collaborators, NIH: Charles Bolan (DTM, CC)
Susan Leitman, M.D. (DTM, CC)

Total Staff Years: 1

Human Research: Human subject research

Keywords: Magnesium Metabolism

Summary: The secretion of parathyroid hormone (PTH) by the parathyroid gland is directly regulated by extracellular calcium ions (Ca2+) via the Ca2+–sensing receptor. However, there are reports of the effect of magnesium (Mg2+) on PTH secretion: acute hypermagnesemia can suppress PTH, and it is suggested that the mechanism of suppression is similar to that of hypercalcemia. During platelet apheresis, the infused citrate forms complexes with both Ca2+ and Mg2+ ions and, therefore, decreases the concentration of the bioactive forms ("ionized" Ca and Mg) of both cations in the blood returned to the donor. The resulting hypocalcemia and concomitant hypomagnesemia could influence the PTH response and could be responsible for the citrate toxicity symptoms observed during apheresis. We investigated the time course of changes in the concentrations of ionized Mg (iMg) and ionized Ca (iCa), and other electrolytes that occurred during plateletpheresis procedures in seven healthy donors undergoing three 90-minute procedures each at fixed citrate-infusion rates. Marked, progressive increases in serum citrate concentration occurred during the rapid infusion of citrate, were accompanied by symptomatic decreases in iCa and iMg and significantly increased renal excretion of calcium, magnesium, and citrate.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010220-09

LBC: DLM

Title: Histidine-rich Glycoprotein: Characterization and Clinical Significance Studies

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: McDonald Horne, III

C*ollaborators, Lab:* Ann M. Cullinane (HEME, CC)
Paula K. Merryman (HEME, CC)

Total Staff Years: .5

Human Research: Human cells or tissues

Keywords: Histidine-rich, Glycoprotein, Fibrinolysis, Platelets, Heparin

Summary: Histidine-rich glycoprotein (HRGP) is a multifunctional plasma protein of unclear physiologic significance. It binds not only proteins (plasminogen, fibrinogen, thrombospondin, vitronectin, immunoglobulin G, complement components), but also heparin, transition metals, and heme, and it binds to several types of cells (T-lymphocytes, macrophages, platelets). Therefore, it may be a modulator of fibrinolysis, an immunoregulator, and a carrier of trace metals. The interaction of HRGP with platelets and the ultimate effect of this interaction on platelet-dependent processes in fibrinolysis are areas of interest. A method for purifying HRGP from fresh plasma was previously developed, and recently we have been using the protein in a variety of studies. So far we have established that HRGP binds saturably to platelets when it is liganded to a transition metal (e.g., zinc) and that this binding is completely blocked by a monoclonal antibody to CD36. Individuals lacking CD36 on their platelets are being sought to confirm the observation that HRG/zinc binds to CD36. We have also demonstrated that the addition of HRGP and zinc to normal plasma can inhibit clinically relevant concentrations of heparin, suggesting that HRGP plus zinc might be useful as a heparin antidote.

LBC: DLM

Title: Identification of Molecular Defects in Patients with Von Willebrand's Disease

Dates: from: 10/01/2001 to: 09/30/2001

Lead Investigator: Margaret E. Rick, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Dennis M. Krizek (HEME, CC)

Total Staff Years: .9

Human Research: Human subject research

Keywords: von Willebrand's Disease

Summary: One family that has an abnormal von Willebrand factor (vWf) with a defective binding site for factor VIII has been studied, and the genetic defect has been identified. The binding defect was initially evaluated by assessing the ability of the patient's vWf to bind purified factor VIII. DNA was purified from peripheral blood leukocytes, specific regions of the vWf gene were amplified by polymerase chain reaction, and direct sequencing of the DNA was carried out. A transition of nucleotide 2451 (T to A) was found, which results in the substitution of GLN for HIS at amino acid 54 in the mature vWf subunit. We recently used a PCR mutagenesis technique to insert the mutation into cloned DNA and have expressed the abnormal protein. The latter was tested in an assay for binding factor VIII and was shown to manifest decreased binding of factor VIII. A manuscript containing the expression data is in preparation. Two unrelated patients with von Willebrand's disease and an abnormal distribution of vWf multimers have been studied, and one new mutation in the A1 region of the vWf gene has been identified in one family. The mutation was cloned into an expression vector and the expressed abnormal vWf is being characterized. The second family is being studied and appears, in preliminary studies, to have a previously unidentified mutation also in the A1 domain of vWf.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010244-07

LBC: DLM

Title: Survival of *Microsporidia* after Exposure to Disinfectants and Environmental Conditions

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Daniel Fedorko

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Nancy A. Nelson (MICRO, CC)

Collaborator, NIH: Theodore Nash (LPD, NIAID)

Total Staff Years: .15

Human Research: Neither human cells nor tissues

Keywords: Microsporidia, Disinfectants

Summary: Microsporidia are ubiquitous parasites causing infections in insects, fish, and mammals. Recently, *microsporidia* have been demonstrated to infect humans. These organisms cause ophthalmic and gastrointestinal infections, primarily in patients with AIDS. Several genera of human pathogens have been cultivated in cell culture. Presently there are no data regarding the ability of the human pathogens *Encephalitozoon intestinalis* and *E. hellem* to survive under various environmental conditions. Also, there are no reports regarding the effects of disinfectants on spores of these two species. The survival of microsporidial spores after exposure to disinfectants such as chlorine, alcohol, and quaternary ammonium compounds and environmental conditions such as elevated temperature and dessication will be studied. Cultivation of *microsporidia* in the shell vial system using various cell lines has been investigated, and *microsporidia* appear to replicate in both fibroblast and epithelial cell lines. A monoclonal antibody produced by Dr. T. Nash (NIAID, LPD) has replaced Giemsa staining for detection of infected cells. Although the monoclonal antibody makes detection of infected cells easier, the assay is still cumbersome and tedious. In an effort to replace staining of infected cell cultures, a method for quantitation of microsporidial DNA has been developed. This assay uses polymerase chain reaction (PCR) and FRET probes in the Light Cycler Instrument to provide real-time PCR data. This assay will provide us with a rapid and sensitive method to replace visual examination of infected cells using the monoclonal antibody.

LBC: DLM

Title: Use of Polymerase Chain Reaction and Restriction Fragment Length Polymorphism Analysis for Identification of *Mycobacteria* and *Nocardia* Species

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Frank G. Witebsky, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Patricia S. Conville (MS, CC)
Steven Fischer, M.D., Ph.D. (DLM, CC)

Total Staff Years: .8

Human Research: Neither human cells nor tissues

Keywords: Polymerase Chain Reaction, Restriction Fragment Length Polymorphism, Polymorphism, *Mycobacteria*, *Norcardia*

Summary: Polymerase chain reaction amplification of a portion of the genome of both rapidly growing *Mycobacteria* and *Nocardia* species, followed by restriction fragment length polymorphism analysis of the amplification products, has proven to be a useful technique in the diagnostic laboratory. Identification of these organisms at the species level can be obtained within a few days of organism isolation, as compared with the month or more required for conventional identification based on biochemical testing. In addition, these molecular procedures allow more accurate discrimination among species and subspecies than is possible with biochemical testing. Our work with two different areas of the *Nocardia* genome (a portion of the gene for 16S ribosomal RNA and a portion of the gene for the heat-shock protein) has suggested the existence of hitherto unrecognized *Nocardia* species; work is ongoing to characterize these organisms further. In addition, we have found some species of *Nocardia* to be human pathogens that were not previously reported to cause disease in patients in the western hemisphere, or were not known to be human pathogens at all, so far as we know. A manuscript describing our methodology has been published. We are currently writing up some of our findings on the unusual *Nocardia* species that we have found to be agents of disease in man.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010265-06

LBC: DLM

Title: Development of a Polymerase Chain Reaction Procedure for Quantitative Measurement of Cytomegalovirus in Blood

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Steven Fischer, M.D., Ph.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, NIH: John E. Bennett (LCI, NIAID)
Karoll Cortez (NIAID)
Gary A. Fahle (MS, CC)
Victoria A. Silcott (MS, CC)

Total Staff Years: .3

Human Research: Neither human cells nor tissues

Keywords: Polymerase Chain Reaction, Cytomegalovirus

Summary: Cytomegalovirus (CMV) disease is a relatively frequent, and often serious, complication in immunocompromised, CMV-infected patients. In the past few years, it has become apparent that to differentiate between subclinical viral shedding and large-scale viral replication, occurring during the prodrome before the onset of active disease, it is necessary to use sequential monitoring with a quantitative assay. Several studies have shown that CMV quantitative polymerase chain reaction (PCR) assays are more sensitive than buffy coat CMV antigen detection assays. This extra sensitivity can, in some cases, give an additional week of warning before the onset of CMV disease. Instituting antiviral therapy earlier in the prodromal stage may decrease the chance of the patient developing active CMV disease. We have completed development of a competitive quantitative PCR assay for the detection of CMV in buffy coat cells. The assay can detect as few as three to five viral genome equivalents in an amplification reaction tube. The coefficient of variance of this assay is about 40 percent, in line with other published descriptions of assays of this type. To have an assay with improved precision and, therefore, better potential predictive value for disease onset or progression, we have developed a real-time CMV PCR assay. This assay utilizes frequency resonance energy transfer fluorescence probes and is designed to run on the Roche LightCycler. Amplification and detection of the assay can be completed within 45 to 50 minutes. In addition to continuing the developmental work on a real-time PCR assay, we have begun performing an evaluation of the Organon Teknika NASBA pp67 CMV assay. This commercially available assay amplifies CMV RNA in an isothermal reaction using reverse transcriptase and RNA polymerase. A retrospective study was conducted comparing the performance of the real-time quantatitive CMV PCR assay with the NASBA pp67 and pp65 antigen detection assays. The real-time CMV PCR assay was found to detect all the significant episodes of CMV viremia identified by the pp65 antigen assay but also gave a positive signal at least a week earlier in about 75 percent of the episodes. The pp67 NASBA assay was less sensitive than the other two methods. These results were presented at the American Society for Microbiology meeting in May 2001. We have initiated a prospective study utilizing the real-time CMV PCR assay to test whole blood samples from bone marrow transplant patients.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010269-06

LBC: DLM

Title: Markers of Disease Activity in Idiopathic Inflammatory Myopathy

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Margaret E. Rick, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Ann M. Cullinane (HEME, CC)
Paula K. Merryman (HEME, CC)

Collaborator, NIH: Paul H. Plotz, M.D. (ARB, NIAMS)

Collaborator, Extramural: Lisa Rider, Food and Drug Administration

Total Staff Years: .15

Human Research: Human subject research

Keywords: Idiopathic Inflammatory Myopathy

Summary: This study is designed to aid in the evaluation of clinical disease activity in patients with idiopathic inflammatory myopathies, a diverse group of diseases that includes inflammation in skeletal muscle. Since the pathology includes primary muscle capillary endothelial cell damage, we have assessed markers of activation and injury to endothelial cells and activation of coagulation factors, including complexes of thrombin-antithrombin, plasmin-antiplasmin, tPa, and thrombomodulin. We have studied 38 patients and are currently analyzing the data to determine clinical correlations with disease activity. A subset of patients shows an increase in thrombin-antithrombin complexes, which is a sensitive assay for activation of coagulation factors. This project is awaiting input from investigators in NIAMS. Further testing is being planned.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010278-05

LBC:	DLM
Title:	Polymerase Chain Reaction–Single-strand Confirmation Polymorphism for the Detection and Speciation of *Microsporidia* in Clinical Specimens
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Daniel Fedorko
Supervisor of Record:	Thomas A. Fleisher, M.D.
Collaborator, Lab:	Nancy A. Nelson (MICRO, CC)
Collaborators, Extramural:	Donna Bertucci, Department of Microbiology, Tulane University Elizabeth S. Didier, Ph.D., Department of Microbiology Tulane University
Total Staff Years:	.2
Human Research:	Neither human cells nor tissues
Keywords:	Polymerase Chain Reaction, *Microsporidia*

Summary: There are many options for the detection and speciation of *microsporidia* in clinical specimens. Light microscopy allows detection of the parasites but does not allow speciation. Electron microscopy is the gold standard for speciation but is not as sensitive a method for the detection of *microsporidia*. Polymerase chain reaction (PCR) is a sensitive technique with many different methods for confirming a positive result and for determining the genus and species causing an infection. Speciation can be achieved by using species-specific primers in the PCR assay, or by using DNA probes or restriction endonucleases to determine the species after the PCR assay is performed. Single-strand confirmation polymorphism (SSCP) combined with PCR (PCR-SSCP) has been used to identify bacteria, fungi, and viruses. We will use our PCR assay for the detection of *microsporidia* in stool specimens and apply SSCP to determine the specific genus and species of the parasite. Organisms from cell culture will also be used to validate the method. The project has been expanded to apply SSCP to the detection of microsporidial genotypes in epidemiologic studies. Newly published primers allow speciation when the PCR products are digested with restriction enzymes. SSCP analysis will make this task easier and more sensitive than restriction enzyme analysis. We have identified two different genotypes among the enterocytozoon bieneusi detected in patients with microsporidiosis at the NIH, which will be used to finish this study. This project has been completed and has been published in the *American Journal of Tropical Medicine and Hygiene* (Fedorko, D .P., Nelson, N.A., Didier, E.S., Bertucci, D., Delgado, R.M., and Hruszkewycz, A.M., 2001. Speciation of human *microsporidia* by PCR–single-strand conformation polymorphism. *Am J Trop Med Hyg* 65:397-401).

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010279-05

LBC: DLM

Title: Comparison of Microbiologic and Cytologic Results for Bronchoalveolar Lavages

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Frank G. Witebsky, M.D.

Supervisor of Record: Thomas A Fleisher, M.D.

Co*llaborators, Lab:* Vee J. Gill, Ph.D. (MS, CC)
Frida Stock, B.S. (MS, CC)

Total Staff Years: .05

Human Research: Human subject research: Interviews

Keywords: Bronchoalveolar Lavages

Summary: Bronchoalveolar lavage specimens are usually split between cytology and microbiology laboratory analysis. Because different methodologies are used by these two laboratories in the workup of these specimens, we thought it would be useful to review the results obtained on these specimens by each laboratory. Such a review might help define the relative sensitivities of the different procedures used, suggest areas of redundancy that might be candidates for elimination, and help identify the procedures most likely to produce clinically significant results. Results from the data analyzed thus far indicate that cytology preparations are more sensitive for the direct detection of significant fungal pathogens than the smears prepared in microbiology, presumably because of the larger volume of material used for preparation of smears in cytology. The data for approximately 7 years have been collected and partially analyzed to assess the relative sensitivities of the procedures performed in the two laboratories, not only for fungi but also for the detection of other pathogens such as *Mycobacterium* tuberculosis and *pneumocystis carinii*. Further analysis of the data has been temporarily postponed to deal with more pressing projects; we hope to complete the analysis within the next year, perhaps with the inclusion of more recent data.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010281-05

LBC: DLM

Title: Detection and Identification of Mycobacteria in Clinical Specimens

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Steven Fischer, M.D., Ph.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, NIH: Gary A. Fahle (MS, CC)
Victoria A. Silcott (MS, CC)

Collaborators, Extramural: Mark Manak, Ph.D., BBI-Biotech Research Laboratories, Inc.
Jang Rampal, Ph.D., Advanced Technology Center, Beckman Coulter, Corp.

Total Staff Years: .3

Human Research: Neither human cells nor tissues

Keywords: Mycobacteria, Clinical Specimens

Summary: Detection and identification of acid-fast bacilli of *Mycobacterium* species by conventional procedures requires growing the organisms from patient specimens and then testing the isolates for various phenotypic characteristics. These methods may take from a period of days to 1 or more months. The development of a few highly specific molecular probes for testing cultures growing acid-fast bacilli has greatly reduced the time to identification of some Mycobacterial isolates. Recently, the polymerase chain reaction and isothermal nucleic acid amplification techniques have been used in assays that offer a high degree of specificity and reasonable sensitivity for detection of *Mycobacterium* tuberculosis in clinical samples. At present, no commercially available amplification assay systems are capable of detecting multiple *Mycobacterium* species while excluding cross-reactive signals from other bacteria commonly present in clinical samples. Experiments have been successfully performed with a version that simultaneously detects the presence or absence of nucleic acid amplification products from six common clinically isolated *Mycobacterium* species. A joint patent application between the NIH and Beckman Coulter Corporation has recently been submitted. Because sample preparation is a critical component of molecular diagnostic assays, we have initiated a new effort to investigate the usefulness of a pressure cycling technology (PCT) for improving the efficiency of nucleic acid release from *Mycobacterium* cells. A barocycler (device for pressure cycling) has been recently brought into CPD under a CRADA with BBI Biotech. With a device on site, further experiments with pressure cycling performed on cultures of different *Mycobacterium* species were initiated. Using PCT as a pretreatment before nucleic acid extraction, we were able to increase the average PCR signals from suspensions containing *Mycobacterium gordonae* and suspensions of *Mycobacterium* tuberculosis.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010283-05

LBC: DLM

Title: Platelet-associated Antibodies in Patients with Autoimmune Thrombocytopenic Purpura

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Margaret E. Rick, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Kristen Hansmann (HEME, CC)

Total Staff Years: .2

Human Research: Human subject research

Keywords: Platelet-associated Antibodies, Autoimmune Thrombocytopenic Purpura

Summary: Autoimmune (idiopathic) thrombocytopenic purpura (ITP) is a disease caused by autoantibodies directed against platelets, but the demonstration of specific antibodies has been difficult for a variety of reasons. In general, when the antibodies can be demonstrated, there is an inverse correlation with the platelet count. We have set up an assay for specific platelet glycoproteins, to aid in the diagnosis, treatment, and monitoring of patients with ITP. We will use the tests particularly for the followup of patients before and after treatment in a study with NHLBI in the treatment setting of T-cell-depleted auto-stem cell transplantation in patients with severe ITP. Sixteen patients have been studied. A verbal presentation will be given at the national meeting of the American Society of Hematology in December 2001.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010286-05

LBC: DLM

Title: Assessment of Peripheral Blood Monocytes in Patients with Recurrent Mycobacterial Infection

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Thomas A. Fleisher, M.D.

Collaborator, Lab: Margaret R. Brown, M.A. (IMMUNE, CC)

Collaborators, NIH: Steven M. Holland, M.D. (LHD, NIAID)
Gulbu Uzel, M.D. (LHD, NIAID)

Total Staff Years: .3

Human Research: Human subject research: cells or tissues

Keywords: Blood Monocytes, Mycobacterial Infection

Summary: Monocytes are being characterized for the expression levels of CD40, CD80, CD86, CD120b, and the gamma interferon receptor alpha chain. These studies have identified two patients with gamma interferon receptor alpha chain deficiency, and the molecular level of this defect is being actively examined. In addition, interferon gamma receptor beta chain deficiency has also been characterized in one patient at the molecular level. As a consequence of these studies, an intracellular flow cytometric method for evaluating STAT protein phosphorylation has been developed and validated initially for STAT1 and more recently for STAT4. The combination of cell surface receptor and functional status at the level of cytokine response is being applied to new patients and is also being used to evaluate patients after bone marrow transplantation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010287-05

LBC: DLM

Title: Assessment of Lymphocytes in Patients with Autoimmune Lymphoproliferative Syndrome

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Thomas A. Fleisher, M.D.

Collaborator, Lab: Margaret R. Brown, M.A. (IMMUNE, CC)

Collaborator, NIH: Stephen E. Straus, M.D. (LCI, NIAID)

Collaborator, Extramural: Jack Bleesing, M.D., Pediatrics, Arkansas Children's Hospital Research Institute

Total Staff Years: .3

Human Research: Human subject research: cells or tissues

Keywords: Autoimmune Lymphoproliferative Syndrome

Summary: An extensive flow cytometric evaluation continues of patients with autoimmune lymphoproliferative syndrome (ALPS) and their extended family members, on the basis of characterization of the expanded double-negative T-cell and B-cell populations. Double-negative T cells have been demonstrated to be alpha beta TcR, CD57+, HLA-DR+, and CD45RA+. This study has been extended to characterize the double-negative T cells more completely, including B220 expression and gamma-delta TcR T cells in all ALPS patients. In addition, we have initiated expanded characterization of the B cells, directed at memory B cells using CD27 and B220 assessment in these patients. More recently, we have identified a relative deficiency in CD4/CD25 T cells that could be associated with the autoimmunity in this disorder based on recent information identifying this T cell as a critical immunoregulatory T cell. Functional studies directed at this T-cell subpopulation will be initiated in the near future.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010290-04

LBC: DLM

Title: Genetic Abnormalities in Patients with Thrombophilia

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Margaret E .Rick, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Kristen Hansmann (HEME, CC)

Total Staff Years: .25

Human Research: Human subject research

Keywords: Thrombophilia, Genetic

Summary: Patients with recurrent venous thromboembolic disease, thrombotic disease at an early age, and/or a family history of this disease are at higher risk for recurrences of thrombosis; their family members are also potentially at higher risk than healthy subjects. These patients are being studied for genetic abnormalities that may predispose them to thrombosis, including abnormalities in the factor V gene (factor V Leiden), prothrombin gene abnormality 20210, and the mutation leading to labile 5, 10 methylenetetrahydrofolate reductase (which increases plasma levels of homocysteine, leading to thrombosis). Postmenopausal women who take hormonal replacement therapy may also be at risk for thrombotic disease, and selected subjects are being screened. DNA is isolated from peripheral blood leukocytes, and the DNA is analyzed using polymerase chain reaction and restriction enzyme techniques. More than 170 patients have been studied thus far for these abnormalities.

LBC: DLM

Title: Evaluation of an Alternative Pathway for Class I MHC Product-dependent Presentation of Oligopeptide Antigens

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Roger Kurlander

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Elizabeth S. Chao (HS, CC)
Janet R. Fields (HS, CC)
Abdul Tawab (HS, CC)

Collaborators, NIH: Jay A. Berzofsky, M.D., Ph.D. (MIVRS, NCI)
Ciriaco A. Piccirillo, Ph.D. (LI, NIAID)

Total Staff Years: 3

Human Research: Neither human cells nor tissues

Keywords: Class I MHC Product-dependent, Oligopeptide Antigens, Vaccines, Tetramers, Costimulation

Summary: There is ongoing interest in using antigen-specific CD8 cells induced by vaccines to treat infectious, neoplastic, and transplantation-related diseases. One major obstacle has been difficulty in generating and maintaining strong CD8 responses in vivo. To dissect the relationship between antigen presentation and antigen-specific CD8 T cell responses, my lab has been studying the murine response to the pathogenic bacterium Listeria monocytogenes (LM), and especially to the LM product, lemA. LemA contains a highly immunogenic aminoterminal fragment, f-MIGWII, which can be presented to CD8 cells by the MHC class Ib product H2M3. In prior years, I have cloned and purified a recombinant form of lemA (r-lemA), confirmed that it can be effectively processed and presented to CD8 T cells *in vitro*, and demonstrated that lemA-treated mice generate large numbers of f-MIGWII-specific CD8 T cell in response to inoculation. In our more recent studies, we use lemA as a tool to study immune CD8 T cell generation and function. To assess the protective potential of lemA-immune cells *in vivo*, during the past year we have examined host resistance against LM in lemA-treated animals. We found that inoculated mice have markedly increased antilisterial immunity 7 days post-inoculation but do not retain this resistance one month later, even though they still have substantial numbers of lemA-immune CD8 memory cells. The reasons for this discrepancy remain as yet unclear. These studies demonstrate that some subsets of microbe-specific CD8 cells have surprisingly selective roles in the host defense. In this case, f-MIGWII-immune cells appear to contribute significantly to the containment of primary disease but not to the maintenance of long-term memory. Prospective screening for such selectivity in function may be a valuable precaution whenever potential immunogens are being considered for possible use in antimicrobial vaccines. To examine the impact of excess free immunogenic peptides on CD8 T cell responses, we have inoculated mice with mixtures of lemA and f-MIGWII,

the active immunogenic peptide recognized by lemA-immune CD8 cells. Although infusions of f-MIGWII alone do not produce measurable responses, mice inoculated with an equimolar mixture of f-MIGWII and lemA demonstrate only one-third of the expected normal lemA-immune response, and the lemA immune cells generated show reduced avidity for f-MIGWII *in vitro*. These findings have several practical implications. First, they demonstrate that overly aggressive use of immunogenic antigens *in vivo* can adversely affect immune CD8 responses. Conversely, these studies suggest that short-term, high-dose peptide treatment at the time of immune stimulation may be a useful approach for selectively downregulating subsets of antigen-specific effectors *in vivo*. Such a strategy may be valuable, for example, in probing the physiologic role of epitope-specific CD8 subsets in host defense against pathogens. H2M3-restricted lemA-immune T cells are an atypical CD8 subpopulation. To study lemA-like products and other antigens in a more conventional model system, we have generated a lemA-like construct (lemS) that contains a model immunogenic peptide Ova257-264, derived from ovalbumin, inserted immediately adjacent to the backbone of lemA. The resulting construct is processed by APC with the release of Ova257-264, an immunogenic peptide that is readily presented to CD8 cells by the class Ia MHC product H2Kb. Using a series of recently published assays and reagents (including MHC-peptide tetramers), we have compared antigen presentation by CD8 T cells in the days immediately after inoculation with the ovalbumin, ovalbumin in incomplete Freund's adjuvant (IFA), and lemS, and assessed the ultimate CD8 response to inoculation 7 days later. At equal doses ovalbumin, infused subcutaneously *in vivo* produces a different pattern of antigen presentation and CD8 T cell response than ovalbumin/IFA and lemS. The ova257-264 element in ovalbumin is presented *in vivo* quite strongly. By contrast, Ova257-264 presentation is barely detectable after inoculation with ova/IFA or lemS. Despite lower antigen presentation, ovalbumin/IFA and lemS each elicit five- to tenfold greater CD8 responses *in vivo* than soluble ovalbumin, demonstrating clearly that even very low levels of antigen presentation can be sufficient to stimulate an extensive CD8 response. On the other hand, extensive antigen presentation alone clearly is not sufficient to ensure a brisk CD8 response. Presumably concurrent "costimulation" (provided, for example, by IFA or the lemA7-33 backbone) must be an important factor in explaining the differences in CD8 responses. In addition, we speculate that the failure of ovalbumin to stimulate a CD8 response could reflect in part active suppression of potential by the relatively large concentrations of immunogenic peptide generated *in vivo*. If so, the ability of IFA or the lemA7-33 backbone to prevent excessive antigen presentation may be a significant factor in explaining their adjuvant activity *in vivo*. During the coming year, we are planning to pursue studies in two areas. (1) Using the lemA model system, we will to examine the mechanisms responsible for the peptide-induced downregulation immune CD8 responses *in vivo* and examine strategies for both maximizing and preventing this effect. The findings may have valuable implications both in vaccine development and in developing methods to induce CD8 T cell tolerance *in vivo*. (2) Using Ova257-264-based antigens, we plan to continue our studies of the relationship between antigen presentation and CD8 responses *in vivo*. Using active LM infection as a model, we will examine the interrelationship between "costimulation," and antigenic peptide presentation in determining the fate of naive and immune CD8 T cells *in vivo*. We hope that these studies will advance efforts to develop a more rational basis for vaccine-mediated manipulation of CD8 cell responses *in vivo*.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010293-04

LBC: DLM

Title: Experimental Treatment of Transfusion-dependent 5q Minus Syndrome with Leucovorin

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Candido Edgardo Rivera, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Total Staff Years: .3

Human Research: Human subject research: cells or tissues

Keywords: Transfusion-dependent 5q Minus Syndrome, Leucovorin

Summary: The objective of this protocol is to determine whether leucovorin treatment can normalize hematopoietic cell growth and differentiation in patients with 5q- syndrome which may lack the gene for dihydrofolate reductase (DHFR) enzyme. The patients will be treated with oral leucovorin for 3 months and will be monitored for improvements in their counts. In addition, bone marrow colony assays will be performed in the presence and absence of leucovorin. The DHFR gene will be sequenced in each patient to screen for DHFR mutations. FISH analysis for the DHFR will also be performed on the cytogenetic material to assess the presence and copy number of the DHFR gene.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010294-04

LBC: DLM

Title: Heparin Cofactor Ii Levels in Patients with Paroxysmal Nocturnal Hemoglobinuria

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Candido Edgardo Rivera, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Total Staff Years: .5

Human Research: Human subject research: cells or tissues

Keywords: Paroxysmal Nocturnal Hemoglobinuria, Heparin Cofactor II Levels

Summary: Thromboembolic events are the most common cause of mortality in patients with paroxysmal nocturnal hemoglobinuria (PNH). Low plasma heparin cofactor II (HCII) levels have been shown to occur in a variety of hemolytic conditions, including thalassemia intermedia and sickle cell disease. The level of HCII is related to the degree of hemolysis. A correlation between low HCII levels and thrombosis has been demonstrated in some of these patients. Because of the association between PNH and thrombosis, we are exploring the possible association between HCII levels and PNH. We have already demonstrated that PNH patients have low normal baseline levels of HCII. We will now focus our efforts on measuring HCII in patients with a significant history of thrombosis. Other coagulation parameters including ATIII, Prot C, Prot S, APCR, Prothrombin 20210, and Methylenetetrahydrofolate polymorphisms will also be measured.

LBC: DLM

Title: Resistance to Multiple Fluoroquinolones in a Strain of *Streptococcus pyogenes*: Detection of Point Mutations

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Daniel Fedorko

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Vee J. Gill, Ph.D. (MICRO, CC)
Nancy A. Nelson (MICRO, CC)
Steve Yan, Ph.D. (MICRO, CC)

Collaborator, NIH: Steven M. Holland, M.D. (LHD, NIAID)

Total Staff Years: .2

Human Research: Neither human cells nor tissues

Keywords: S*treptococcus pyogenes*, Multiple Fluoroquinolones

Summary: A strain of *Staphylococcus pyogenes* (NIH-R01-GAS) was isolated from the blood of an 18-year-old patient with Jobs syndrome who had recently received various antibiotic treatments including levofloxacin. Susceptibility testing revealed that the isolate was resistant to the fluoroquinolone antibiotics. Quinolone resistance-determining regions (QRDRs) of both the parC subunit of topoisomerase and DNA gyrase A are conserved in a number of bacteria belonging to different genera. We designed polymerase chain reaction (PCR) primers flanking the QRDRs of these two genes from *S. pyogenes* on the basis of the amino acid sequence homologies among *S. pneumoniae*, *E. coli*, and *S. aureus*. The amino acid sequences deduced from the amplified gene products from *S. pyogenes* demonstrated significantly high homology to the same regions of these two genes in *S. pneumoniae*. Compared with the quinolone-sensitive ATCC strain, the quinolone-resistant isolate of *S. pyogenes* presented point mutations in DNA gyrase A and in the parC subunit of topoisomerase IV. Recently, we acquired another clinical strain of *S. pyogenes* (NIH-R02-GAS) with increased MICs to some of the fluoroquinolone antibiotics. Amino acid sequences of ParC and GyrA of this isolate demonstrated point mutations only slightly different from those of the first isolate, with serine-79 of the ParC changed to alanine rather than tyrosine, and no mutation of serine-81 of GyrA. Mutation of methionine-99 to leucine was common to GyrA of both isolates. We propose that the minor change in point mutations in the GyrA and uncommon point mutation in the parC gene in strain NIH-R02-GAS may explain the striking differences in fluoroquinolone susceptibilities in these two isolates of *S. pyogenes*. Sequencing of other regions will be performed. This project has been concluded and published in *Antimicrobial Agents and Chemotherapy* (Yan, S.S., Fox, M.L., Holland, S.M., Stock, F., Gill, V.J., and Fedorko, D.P., 2000.) Resistance to multiple fluoroquinolone antibiotics in a clinical isolate of *Streptococcus pyogenes*: Identification of the GyrA and ParC genes and specification of the point mutations responsible for the resistances. *Antimicrob Agents Chemother* 44:3196-3198).

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010300-03

LBC: DLM

Title: Identification of Proteolytic Activity for von Willebrand Factor

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Margaret E. Rick, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: David Aronson (HEME, CC)
Dennis M. Krizek (HEME, CC)

Collaborators, Extramural: Stephan Moll, University of North Carolina School of Medicine
Mark Taylor, University of North Carolina School of Medicine

Total Staff Years: .8

Human Research: Human subject research

Keywords: Proteolytic Activity, von Willebrand's Factor

Summary: Proteolysis of von Willebrand's factor (vWF) normally occurs through the action of a plasma enzyme that has recently been characterized; it accounts for the small quantities of cleavage products normally present in the circulation, and its inhibition can lead to the disease called thrombotic thrombocytopenic purpura (TTP). We have developed a better and more rapid assay to evaluate the cleavage of vWF and have characterized patients with a TTP-like syndrome to detect those with low vWF cleaving protease activity. The assay does not require specialized reagents and can be completed within 6 to 8 hours on patient plasma. We are working on a purification process to isolate plasma vWF protease and better characterize its function.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010302-03

LBC: DLM

Title: Development and Diagnostic Use of Rapid Immunoassays

Dates: from 10/01/2001 to 12/30/2001

Principal Investigator: Alan T. Remaley, M.D., Ph.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Total Staff Years: .25

Human Research: Human cells or tissues

Keywords: Rapid Immunoassays

Summary: Several endocrine tumor markers are routinely used in the diagnosis and management of various cancers. The current assays, however, typically take several hours to perform, which precludes their use in the intraoperative management of patients. Most endocrine tumor markers have a half-life in the circulation of less than 5 minutes, thus making it feasible, if a rapid assay were available, to monitor the concentration of the hormones during surgery or localization procedures. The primary indication for such assays would be to assess the extent of residual tumor after surgery and to localize tumors by selective venous or arterial sampling. In the past year, we have improved the assay for PTH by using antibodies that are specific for the intact protein. We also have modified an existing commercial assay for gastrin for developing a rapid immunoassay. In the upcoming year, we plan to further develop the gastrin assay and to assess its potential clinical utility on patient specimens.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010303-03

LBC: DLM

Title: Development of New Assays for Lipoprotein Testing

Dates: from: 10/01/2001 to: 12/30/2001

Principal Investigator: Alan T. Remaley, M.D., Ph.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Total Staff Years: .25

Human Research: Neither human cells nor tissues

Keywords: Lipoprotein Testing

Summary: Lipoprotein fraction analysis is a valuable tool in estimating the risk for coronary artery disease. The current procedure, however, requires multiple tests and has several manual steps. In order to reduce the complexity and cost of lipoprotein fraction analysis, we have developed a single-tube homogenous assay for measuring serum high-density lipoprotein (HDL) cholesterol, total cholesterol, and triglyceride. Low-density lipoprotein (LDL) cholesterol can then be calculated from these parameters using the Friedewald equation. The assay uses an anti-apoB antibody to block the reactivity of the reporter enzymes to LDL-cholesterol. The assay is performed in a sequential manner so that after the HDL-cholesterol is determined, a detergent is added to disrupt the antibody complex, which allows the subsequent measurement of total cholesterol. Next, the reporter enzymes for measuring total triglyceride are added. In the past year, we found that the assay was compatible with alternative procedures for blocking HDL, and the assay format also worked for first measuring LDL-C followed by total cholesterol. In the upcoming year, we plan to adapt the assay to an existing commercial assay for HDL-C and potentially transfer the technology to a diagnostic company for commercialization.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010304-03

LBC: DLM

Title: Mutation Analysis of Selected Lymphoid-immune Disorders

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Thomas A. Fleisher, M.D.

Collaborator, Lab: Julie E. Niemela (IMMUNE, CC)

Collaborator, NIH: Jennifer M. Puck, M.D. (IG, GMBB, NHGRI)

Total Staff Years: .5

Human Research: Human subject research: cells or tissues

Keywords: Lymphoid-Immune Disorders

Summary: This project represents an extension of a longstanding series of collaborative studies performed to better characterize and understand immune deficiency. Mutations involving the genes for the common gamma chain (X-SCID) and fas (ALPS) are being evaluated using dideoxyfinger printing (ddF) and direct gene sequencing. These studies have continued to identify a number of new mutations in both diseases, and these data have been published and submitted for publication. In addition, this project has provided valuable experience in the critical approaches to molecular diagnosis of genetic disorders. The procedure manuals and technical approaches are being used to assist with the NIH CLIA resource program in areas of molecular diagnostics.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010305-03

LBC: DLM

Title: Analytical Performance and Clinical Utility of Thyroid Function Tests

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Gyorgy Csako, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Rene A. Costell, M.T. (CCS, CC)
Alan T. Remaley, M.D., Ph.D. (CCS, CC)

Collaborators, NIH: Lynnette K. Nieman, M.D. (PREB, NICHD)
Frank R. Pucino, Jr., Pharm.D. (CC)
Nicholas J. Sarlis, M.D., Ph.D. (CEB, NIDDK)
Monica C. Skarulis, M.D. (DIR, NIDDK)
Robert A. Wesley, Ph.D. (OD, NCI)

Total Staff Years: .3

Human Research: Human subject research

Keywords: Thyroid Function Tests, Thyroid Nodules, Thyroid Cancer, Quantitative Research Synthesis, Meta-analysis, Hill Criteria

Summary: Thyroid diseases represent the most common endocrine abnormalities, and it has been estimated that about 20 million levothyroxine prescriptions are dispensed to about 1.8 percent of the population in the United States annually. Optimal use of valid laboratory tests for the assessment of thyroid status is thus important both medically and economically. In a multi-institute collaborative study, we assessed the performance of thyroid function tests for monitoring and the potential clinical utility of thyroid hormone therapy for suppressing papillary and/or follicular thyroid cancer. The study was carried out by our previously developed method of quantitative research synthesis for assessing the efficacy of thyroid hormone suppression therapy on benign solitary thyroid nodules. This approach involves systematic review of the literature for relevant clinical studies and expert opinions, meta-analysis of selected interventional studies, survey of NIH endocrinology practitioners for therapeutic decisions in hypothetical patients with papillary and/or follicular thyroid cancer, practice validation in NIH patients treated with levothyroxine suppression for papillary and/or follicular thyroid cancer, and application of Hill's criteria to assess causality between thyroid suppression therapy and control of papillary and/or follicular thyroid cancer. In a case study, we investigated the utility of laboratory tests for revealing a clinically important drug interaction. This drug interaction occurred as a result of concomitant ingestion of levothyroxine with high doses of calcium carbonate in the presence of chronic gastrointestinal disorders. Despite different etiologies, initial presentation of this case was similar to our previously described case in which increased doses of levothyroxine replacement therapy were required to achieve euthyroid status in a patient with nephrotic syndrome.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010306-03

LBC: DLM

Title: Analytical Performance and Clinical Utility of Laboratory Tests for Atherothrombosis

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Gyorgy Csako, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Rene A. Costello, M.T. (CCS, CC)
Rosario M. Delgado, M.T. (CCS, CC)
Alan T. Remaley, M.D., Ph.D. (CCS, CC)

Collaborators, NIH: Richard O. Cannon, M.D. (CB, NHLBI)
Susan Leitman, M.D. (DTM, CC)
Frank R. Pucino, Jr., Pharm.D. (CC)
Arshed A. Quyyumi, M.D. (CB, NHLBI)
Silvia M. Santamarina-Fojo, M.D., Ph.D. (MDB, NHLBI)
Robert A. Wesley, Ph.D. (OD, NCI)

Collaborators, Extramural: Stephen E. Epstein, M.D., Cardiovascular Research Institute
Washington Hospital Center
Jianhui Zhu, M.D., Cardiovascular Research Institute
Washington Hospital Center

Total Staff Years: .9

Human Research: Human subject research

Keywords: Atherosclerosis, Thrombosis, HDL, LDL, VLDL, Lipoprotein(a), Infection, Inflammation

Summary: In analytical studies, we developed and evaluated homogeneous assays for sequential testing of up to four lipid/lipoprotein constituents (HDL-cholesterol, apolipoprotein B, total cholesterol, and triglycerides) in a single tube. These new methods are particularly cost-effective in screening for subjects with high cardiovascular risk. In animal studies, we analyzed the laboratory and morphologic abnormalities in lecithin:cholesterol acyltransferase (LCAT) deficient mice. We showed that these animals develop serum lipoprotein X, glomerulosclerosis, and atherosclerosis and are a useful model of human LCAT deficiency. In collaborative clinical studies, we continued studying atherogenic plasma constituents and markers with respect to their analytical and diagnostic performance and overall clinical utility. In a series of studies, we investigated the effects of estrogen, vitamin E, and the selective estrogen receptor modulator raloxifen on vascular responsiveness, serum lipid profile, and inflammatory vascular markers in postmenopausal women. We observed divergent effects of estrogen hormone replacement therapy on serum markers of inflammation in postmenopausal women with coronary artery disease on appropriate medical management. In other studies dealing with the clinical significance of various viral (e.g., cytomegalovirus, herpes simplex viruses, hepatitis A virus) and bacterial (e.g., *Helicobacter pylori*, *Chlamydia pneumoniae*)

infections and inflammatory processes in general, we observed that (a) total pathogen burden affects both coronary artery disease risk and serum C-reactive protein levels, and (b) the host response to cytomegalovirus infection is a determinant of susceptibility to coronary artery disease with gender-based differences in inflammation and type of immune response. Further, we found that antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease, indicating an autoimmune component of atherogenesis.

LBC: DLM

Title: Development and Clinical Application of Molecular Diagnostic Tests

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Gyorgy Csako, M.D.

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Rene A. Costello, M.T. (CCS, CC)
Rosario M. Delgado, M.T. (CCS, CC)
Steven Fischer, M.D., Ph.D. (DLM, CC)

Collaborators, NIH: Adriana Marques, M.D. (LCI, NIAID)
Trey Sunderland, M.D. (NIMH)
Vishakha Thaker, Ph.D. (CC)

Total Staff Years: .5

Human Research: Human subject research: cells or tissues

Keywords: PCR, RFLP, SSCP, DNA, Alzheimer's Disease, Coronary Heart Disease, SLE

Summary: An increasing number of genes have been linked to Alzheimer's disease (AD) over the past decade. Although there is contrary evidence, some recent case-control studies suggested that a C to T polymorphism in exon 2 of the cathepsin D (CATD) gene increases the risk of sporadic AD. The gene encoding cathepsin D is located at the extremity of the short arm of chromosome 11, in the p15 band. The transcribed portion of the gene is about 11,000 bp and is organized into 9 exons analogous with the human pepsinogen A gene. Cathepsin D is a major intracellular aspartyl protease present in the endosomal-lysosomal system. The C to T polymorphism in exon 2 of the CATD gene is common and results in an amino acid sequence change at residue 224 from Ala to Val. The T allele may be associated with increased protein expression (increased pro-cat D secretion) and altered intracellular maturation. We developed new and practicable methods for detection of the C to T polymorphism in exon 2 of the CATD gene. Using newly designed primers and/or a different restriction endonuclease, we markedly shortened the incubation time and reduced the incubation temperature from 60°C to 37°C in conventional PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of DNA extracted from peripheral blood of patients and their controls. Based on PCR-single strand conformation polymorphism (PCR-SSCP) detection, we also developed a semi-automated method. We showed that, under optimized conditions, this method is as reliable as PCR-RFLP and direct DNA sequencing but is simpler and faster to perform. Further, we developed and validated a fully automated, real-time ultrafast PCR method based on the use of the LightCycler instrument for detection of the C to T sequence polymorphism in the CATD gene. With their increased level of automation and speed, the latter two methods would be favor-able replacements for PCR-RFLP and direct DNA sequencing for both routine laboratory use and large-scale screening. Regarding the possible role of infectious agents in the development of AD, our collaborative study showed no increased incidence of herpes simplex type 1 virus (HSV-1) DNA in the brains of patients with AD and apolipoprotein E4 allele, making the participation of HSV-1 in AD unlikely. In other collaborative studies, we continued studying the possible pathogenetic role of apolipoprotein(a) isoforms and alleles in patients with atherosclerotic heart disease and systemic lupus erythematosus.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010308-02

LBC: DLM

Title: Assessment of Memory B Cells in Immune Disorders

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Thomas A. Fleisher, M.D.

Collaborators, Lab: Margaret R. Brown, M.A. (IMMUNE, CC)
Cristin Elizabeth Hill (IMMUNE, CC)

Collaborator, NIH: Peter E. Lipsky, M.D. (AB, NIAMS)

Collaborator, Extramural: Jack Bleesing, M.D., Pediatrics, Arkansas Children's Hospital Research Institute

Total Staff Years: .2

Human Research: Human subject research

Keywords: Memory B Cells, Immune Disorders

Summary: A project to develop the complete immunophenotype of memory B cell has been initiated. This is being done evaluating normal subjects and patients with a number of immune disorders. In addition, the immunophenotypic data are being compared with Ig gene recombination results generated in Dr. Lipsky's laboratory (NIAMS). These investigations have established that CD27 expression is altered in CGD, and this is a direct product of the defective oxidase activity as reflected by the link between CD27 expression and the proportion of normal cells in X-linked carriers. In addition, CD27 expression is markedly diminished in ALPS, primarily related to protein cleavage from the cell surface. The actual link between CD27 expression and B-cell memory status as well as mechanisms that control CD27 expression on B cells is currently under investigation based on the findings in CGD and ALPS.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010309-02

LBC: DLM

Title: Assessment of B220 Expression on Human Lymphocytes

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Thomas A. Fleisher, M.D.

Collaborator, Lab: Amie Elizabeth Bryson (IMMUNE, CC)

Collaborator, Extramural: Jack Bleesing, M.D., Pediatrics, Arkansas Children's Hospital Research Institute

Total Staff Years: .3

Human Research: Human subject research

Keywords: B220 Expression, Human Lymphocytes

Summary: A project to evaluate the CD45 alternative isoform, B220, expression on lymphocytes has been initiated. This is being done in healthy subjects and patients with auto-immune lymphoproliferative syndrome (ALPS) as well as in other patients with lymphocyte disorders. Preliminary data suggest that this isoform is expressed at different levels in certain lymphocyte disorders and may serve as a characteristic finding in subtypes of ALPS. In addition, it appears to distinguish a subset of memory B cells and to be expressed on ono-isotype switched B cells that do not have a memory phenotype.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010310-02

LBC: DLM

Title: *In Vitro* Activities of Fluoroquinolone Antibodies against Blood Culture Isolates

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Daniel Fedorko

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Vee J. Gill, Ph.D. (MICRO, CC)
Steve Yan, Ph.D. (MICRO, CC)

Total Staff Years: .2

Human Research: Neither human cells nor tissues

Keywords: Fluoroquinolone Antibiotics, Steptococci, Bone Marrow Transplantation

Summary: Thirty-seven strains of viridans streptococci isolated from blood cultures on bone marrow transplant (BMT) recipients during the past 4 years and 39 strains of viridans streptococci isolated from non-BMT patients were tested for fluoroquinolone susceptibility. Of the five strains from BMT patients that were resistant to levofloxacin, trovafloxacin, and/or grepafloxacin, one isolate, NIH-(R02), demonstrated high-level resistance to multiple fluoroquinolones tested. DNA gyrase A is one of the major targets for quinolone antibiotics. The amino acid sequence within the quinolone resistance-determining region (QRDR) of the gyrase A of NIH-(R02) was determined. When compared with ten sensitive strains of *S. mitis*, NIH-(R02) carried a single point mutation in the "GKYHPHGDS" box within the QRDR of the gyrA gene (serine to leucine replacement). Thus, the mechanism of fluoroquinolone resistance in this isolate of *S. mitis* is likely similar to the previously reported mechanisms in *S. pneumoniae*. We will sequence the QRDR of the parC subunit of topoisomerase IV (parC gene) of NIH-(R02) and the ten sensitive strains to search for additional point mutations that may confer resistance to the quinolone antibiotics in the viridans group of streptococci.

LBC: DLM

Title: Detection of Toxigenic *Clostridium Difficile* in Stool by Polymerase Chain Reaction

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Daniel Fedorko

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Nancy A. Nelson (MICRO, CC)

C*ollaborator, Extramural:* Charles P Cartwright, Ph.D., Clinical Microbiology
Hennepin County Medical Center

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: *Clostridium Difficile*, Diagnosis, Polymerase Chain Reaction

Summary: *Clostridium difficile*-associated disease (CDAD) is a major problem for hospitalized patients receiving antibiotics or antineoplastic agents. Current laboratory methods for diagnosing CDAD include culture and assays for detection of the toxins produced by the organism or a cell-associated antigen. Polymerase chain reaction (PCR) assays for the diagnosis of CDAD have been reported in the literature, but these reports are limited in scope, and many of the primer pairs used are inefficient or amplify inappropriate portions of the *C. difficile* genome. We have selected five primer pairs specific for the two toxin genes *of C. difficile*. We are in the process of selecting the two primer pairs that are the most efficient in amplifying their targets. We will then develop a real-time PCR using the LightCycler and will use this assay to evaluate the utility of PCR for the diagnosis of CDAD using stool specimens.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010317-01

LBC: DLM

Title: Interaction of Plasminogen with Human Platelets

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: McDonald Horne, III

Collaborators, Lab: Ann M. Cullinane (HEME, CC)
Paula K. Merryman (HEME, CC)

Total Staff Years: 1.5

Human Research: Human cells or tissues

Keywords: Plasminogen, Platelets, Fibrinolysis

Summary: Dissolution of blood clots (fibrinolysis) requires plasmin, a protease derived from the activation of plasminogen by tissue plasminogen activator (tPA). Both plasminogen and tPA are known to bind to the surface of platelets, where their interaction becomes greatly accelerated. Therefore, platelets are important promoters of fibrinolysis. Our laboratory has been examining plasminogen binding to platelets in some detail. We use classical equilibrium binding experiments with the goal of establishing the number of binding sites and the binding affinity. We are also chemically cross-linking biotinylated plasminogen to platelets and then testing for plasminogen-receptor complexes by Western blotting. Our goal is to identify the platelet membrane protein(s) that binds plasminogen to activated and resting cells. The literature indicates that platelet activation enhances plasminogen and that the increased binding is not directly to the platelets but to platelet-bound fibrin. However, our data suggest that there is direct plasminogen binding to platelets, both when they are resting and when they are activated. The number of binding sites appears to be unchanged by activation, but binding affinity is greatly increased. However, the effect of monoclonal antibodies on plasminogen binding to resting and activated platelets is different.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010318-01

LBC: DLM

Title: Development of a Cellular Immune Function Program

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Thomas A. Fleisher, M.D.

Collaborators, NIH: Jennifer M. Puck, M.D. (IG, GMBB, NHGRI)
Gulbu Uzel, M.D. (LHD, NIAID)

Total Staff Years: .5

Human Research: Human subject research: cells or tissues

Keywords: Evaluation, Immune Function

Summary: An approach to evaluate immune function consisting of T-cell proliferative responses to mitogens and recall antigens as well as B-cell responses to recall and neo-antigens is being developed. This is being done in a consistent manner with well-developed controls, and plans to extend these studies into cytokine production patterns are already made. Once fully operational, this will provide critical information in the evaluation of patients with possible immune disorders and in following patients other bone marrow transplantation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010319-01

LBC:	DLM
Title:	Platelet Function in Patients Treated with SSRI Versus Non-SSRI Antidepressants
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Margaret E. Rick, M.D.
Supervisor of Record:	Thomas A. Fleisher, M.D.
C*ollaborator, Lab:*	Donna Jo A. Mayo (HEME, CC)
Collaborators, Extramural:	Teodor Postolache, The National Center for the Treatment of Phobias, Anxiety and Depression Bernard Vittone, The National Center for the Treatment of Phobias, Anxiety and Depression
Total Staff Years:	.1
Human Research:	Human subject research: cells or tissues
Keywords:	SSRI Anti-depressants, Platelet Function

Summary: SSRIs are widely used antidepressant agents, that are known to decrease platelet serotonin content. They have been reported to be associated with bleeding in a minority of patients and recently have been associated with an increase in gastrointestinal bleeding. The purpose of this study is to better understand the potential risks of bleeding associated with mild platelet dysfunction in patients using SSRIs and to determine whether a global test of platelet function, as performed on the platelet function analyzer-100, is able to identify the changes in platelet function associated with SSRI use.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010321-01

LBC: DLM

Title: Macromolecular Substrates for Enzymes

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Glen Hortin

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborator, Lab: Bonnie S. Meilinger, M.T. (CCS, CC)

Total Staff Years: .4

Human Research: Human cells or tissues

Keywords: Thrombosis Coagulation Factors, Thrombin, Proteases, Amylase

Summary: Measurements of enzyme activity serve as important tools for the diagnosis of disease processes and for basic research. We are seeking to develop new substrates for enzymes that improve the ability to measure or the specificity of measuring enzyme activity. We have found that linking small chromogenic and fluorogenic substrates to polymer molecules serves as an approach to prepare substrates with large molecular size. Initial results are described in *Clinical Chemistry* 2001;47:215-222. The molecular size of the substrates is determined primarily by the size of the attached polymer so that it is adjustable. This permits substrate size to be analyzed as an independent variable, and it serves as a means of assessing the steric hindrance of the active sites of enzymes. The large synthetic substrates serve as better models of natural substrates that are of large molecular size, such as the protein substrates of physiological proteinases and the large starch substrates of amylase. Use of large substrates appears to distinguish free protease molecules from those complexed with the inhibitor alpha-2-macroglobulin. Thus, these substrates appear to have utility for the more accurate measurement of the functional activity of plasma proteinases, such as thrombin.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010322-01

LBC: DLM

Title: Homocysteine and Cysteine Interactions

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Glen Hortin

Supervisor of Record: Thomas A. Fleisher, M.D.

Collaborators, Lab: Gyorgy Csako, M.D. (CCS, CC)
Bonnie S. Meilinger, M.T. (CCS, CC)

Total Staff Years: .5

Human Research: Human cells or tissues

Keywords: Thrombosis, Atherosclerosis, Homocysteine, Cysteine

Summary: Increased concentrations of the amino acid homocysteine in blood are recognized as a risk factor for thrombosis and atherosclerosis. Cysteine is a product formed from homocysteine, and increased concentrations of this amino acid may also serve as a risk factor for thrombosis and atherosclerosis. The mechanism of how these amino acids cause or serve as markers for these disorders is not known. Most of these amino acids are bound to albumin or other plasma proteins and mechanisms of exchange of these amino acids are not fully understood. Our studies are examining the distribution of homocysteine and cysteine among plasma proteins and mechanisms of exchange between free and protein-bound forms of these amino acids. The goals of these studies are (1) to identify which measurements of plasma homocysteine, cysteine, or combinations of amino acid measurements serve as the best risk indicator; (2) to better understand the metabolic relationships of homocysteine and cysteine; and (3) to determine whether these amino acids have direct effects on function of plasma proteins.

NURSING DEPARTMENT

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LBC: NURS

Title: Quality of Life in Myeloablative Versus Non-myeloablative Bone Marrow Transplant

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan F. Marden, R.N., Ph.Dc.

Supervisor of Record: Clare E. Hastings, R.N., Ph.D.

Collaborators, Lab: Margaret F. Bevans (NURS, CC)
Georgia J. Cusack (NURS, CC)
Helen S. Mayberry (NURS, CC)
Priscilla V. Rivera (NURS, CC)

Collaborators, NIH: Michael R. Bishop (MB, NCI)
Ronald E. Gress, M.D. (EIB, NCI)

Total Staff Years: .06

Human Research: Human subject research

Keywords: Non-myeloblative Bone Marrow Transplant, Myeloblative Bone Marrow Transplant, Bone Marrow Transplant, Quality of Life, Symptom Distress

Summary: Clinical research in blood stem cell and bone marrow transplantation documents improvements in disease-free intervals, disease-free survival, and the severity of treatment-related toxicities. However, it is important for patients and families to know the quality of life (QOL) they can expect following an allogeneic transplant. The purpose of this study is to describe the QOL experienced by patients undergoing a non-myeloablative allogeneic peripheral blood stem cell transplant and compare it with the QOL experienced by patients undergoing a myeloablative transplant. Patients must be over the age of 18 to enroll. Patients respond to questionnaires that measure QOL and symptom distress using Touch Screen computers. The questionnaires are administered prior to transplant and at set intervals post transplant. Data will be analyzed using multivariate techniques. Forty-eight subjects have been accrued to date. Data collection and subject accrual continue.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001132-02

LBC: NURS

Title: Quality of Life in HIV Patients Receiving Structured Versus Interrupted Treatment

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan F. Marden, R.N., Ph.Dc.

Supervisor of Record: Clare E. Hastings, R.N., Ph.D.

Collaborators, Lab: Rosemary E. McConnell, R.N., B.S.N. (NURS, CC)
April E. Powers, R.N., B.S.N. (NURS, CC)

Collaborators, NIH: Richard T. Davey, M.D. (CRS, NIAID)
Mark Dybul, M.D. (IMS, NIAID)

Total Staff Years: .15

Human Research: Human subject research

Keywords: HIV Patients, Quality of Life, Structured Treatment, Interrupted Treatment, Symptom Distress

Summary: Because of multidrug regimens known as highly active antiretroviral therapy (HAART), HIV infection can now be considered a chronic, manageable disease for many people in the United States. However, these therapies come with complex medication administration regimens and numerous distressing side effects that may affect a person's quality of life (QOL). The purpose of this study is to evaluate the QOL and symptom distress in individuals receiving intermittent versus continuous HAART in the treatment of HIV disease. Thirty-five subjects will be assigned to receive continuous HAART therapy and 35 subjects will receive interrupted therapy. Subjects respond to questionnaires that measure QOL and symptom distress using Touch Screen computers. The questionnaires are administered at seven time periods during the study. Data will be analyzed using multivariate techniques. Fifty-two subjects have been accrued to date. Eleven subjects have completed the study. Data collection continues.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001133-02

LBC: NURS

Title: Quality of Life in Melanoma Patients Receiving Vaccine or with Interleukin-2

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan F. Marden, R.N., Ph.Dc.

Supervisor of Record: Clare E. Hastings, R.N., Ph.D.

Collaborators, Lab: Paula M. Muehlbauer, R.N., M.S.N. (NURS, CC)
Debra Parchan, R.N. (NURS, CC)
Susan A. Gantz, R.N., MS (NURS, CC)

Collaborators, NIH: Schwartzentruber Douglas, M.D. (NCI)
Finkelstein Steven, M.D. (NCI)
Seip Claudia, R.N. (NCI)

Total Staff Years: .15

Human Research: Human subject research

Keywords: Melanoma, Quality of Life, Symptom Distress

Summary: The incidence of melanoma is rising faster than any cancer other than lung cancer in women. The primary treatment for melanoma is surgical resection. However, no universally acceptable standard treatment exists for metastatic disease; and the prognosis of patients with Stage IV melanoma is poor. Therefore, information regarding patients' perceptions of the burden imposed by their disease and treatment may enhance treatment decisions. The purpose of this study is to describe the quality of life (QOL) in patients with metastatic melanoma receiving vaccine alone or with high-dose Interleukin-2 (IL-2) or subcutaneous IL-2. Patients respond to questionnaires that measure QOL and symptom distress. Questionnaires are administered at three time points: prior to, during, and after therapy. Data will be analyzed using multivariate techniques. Seventy-three subjects have been accrued to date. Data collection and subject accrual continue.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001134-02

LBC:	NURS
Title:	Quality of Life in Patients with Heart Disease and Left Ventricular Dysfunction
Dates:	from 10/01/2000 to 09/30/2001
Principal Investigator:	Susan F. Marden, R.N., Ph.Dc.
Supervisor of Record:	Clare E. Hastings, R.N., Ph.D.
Collaborator, Lab:	Claiborne Miller-Davis, R.N., B.S.N. (NURS, CC)
Collaborator, Extramural:	Vasken Dilsizian, M.D., Cardiology University of Maryland Medical Center
Total Staff Years:	.12
Human Research:	Human subject research
Keywords:	Heart Disease, Left Ventricular Dysfunction, Quality of Life, Symptom Distress, Angina

Summary: A majority of the research in patients with chronic ischemic heart disease and left ventricular dysfunction deals with increasing patient survival rates and years. Very little research has focused on patients' perceptions of living with this chronic debilitating disease. The purpose of this study is to assess the health-related quality of life (HRQOL), anginal symptoms, and symptom distress experienced by patients with chronic ischemic heart disease and left ventricular dysfunction. The relationship between underlying cardiac condition, anginal symptoms, symptom distress, and HRQOL will be examined. The trend in HRQOL across time vs. treatment group (medical or surgical management) will be evaluated. Patients respond to questionnaires that measure HRQOL, anginal symptoms, and symptom distress. Underlying cardiac condition will be assessed using exercise thallium imaging parameters and positron emission tomography (PET) imaging parameter (viability). Questionnaires are administered at three time intervals over a 1-year period. Data will be analyzed using multivariate techniques. Twenty-five subjects have been accrued to date. Data collection continues.

LBC: NURS

Title: Technology Dependency and Health-related Quality of Life: A Test of a Model

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan F. Marden, R.N., Ph.Dc.

Supervisor of Record: Clare E. Hastings, R.N., Ph.D.

Collaborator, NIH: Lameh Fananapazir, M.D. (NHLBI)

Total Staff Years: .15

Human Research: Human subject research: Interviews

Keywords: Health-related Quality of Life, Technology Dependency, Implantable Defibrillators, Illness Perceptions, Symptom Distress

Summary: With the efficacy of implantable cardioverter defibrillator (ICD) therapy well established, it is important to understand how ICD recipients perceive their dependence on this life-saving technology and how these perceptions influence their health-related quality of life (HRQOL). The purpose of this study is to test a model that may explain the link between attitudes toward dependency on technology and HRQOL in a sample of adult ICD recipients. The model consists of seven variables: attitudes toward technology dependency, age, gender, illness history, illness representation, symptom distress, and HRQOL. Adult subjects who have received an ICD will be asked to participate. Subjects will complete a questionnaire and return it via the mail. Structural equation modeling techniques will be used to test the model. The hypothesis for this study is that the model fits data from the sample of adult ICD recipients.

REHABILITATION MEDICINE DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL060011-22

LBC: RM

Title: Diagnostic Capabilities of Ultrasound on the Oropharynx and Larynx

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Barbara C. Sonies, Ph.D.

Supervisor of Record: Lynn H. Gerber, M.D.

Collaborators, Lab: Gloria Chi-Fishman, Ph.D. (SLPS, CC)
Jeri L. Miller, Ph.D. (SLPS, CC)

Collaborator, NIH: Carter Van Waes, M.D., Ph.D., (HNSB, NIDCD)

Total Staff Years: .66

Human Research: Human subject research: Interviews and Minors

Keywords: Ultrasound Imaging, Swallowing, Speech, Viscosity, Head Neck Tumors, Three-dimensional Imaging

Summary: The purpose of this project is to evaluate and develop a variety of clinical applications for noninvasive ultrasound imaging to the diagnosis and treatment of impaired swallowing and speech and to evaluate the oropharyngeal structures (tongue, palate, floor muscles, hyoid, larynx, pharynx) in both normal and abnormal populations. This past year we have successfully developed a new three-dimensional (3D) ultrasound system for imaging the oropharynx. This system allows us to systematically track head and neck tumor growth, inflammatory changes in oral tissues, and soft tissue changes in the oropharynx resulting from concurrent radiation therapy, chemotherapy, and surgery in patients with advanced head and neck tumors. We are collaborating with NIDCD and NCI in this application. We have collected long-term recovery (24 to 30 months) and morbidity data on our original 23 subjects and an additional 11 patients with head and neck tumors. The natural evolution of swallowing function and course of recovery of oral motor function and return of eating behaviors is now under study. An outcome matrix is being used to chart dependence/independence during eating, swallowing function, and oral safety. Analysis of the effects of viscosity and volume on hyoid motion in 31 normals revealed that there were significant effects of the thickness of the bolus on hyoid motion. Age and gender differences were also found during swallowing that suggest that anatomical variations, sensory acuity, and muscle force changes occur with normal aging, that can affect swallowing kinematics. We have recently used 3D ultrasound imaging procedures to track postsurgical oral-facial swelling in patients who have had removal of the 3rd molar and find that this technique is a reliable marker.

LBC: RM

Title: A Rigid Body Database on Human Movement

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Steven J. Stanhope, Ph.D.

Supervisor of Record: Lynn H. Gerber, M.D.

Collaborators, Lab: Thomas M. Kepple (BS, CC)
Karen L. Siegel (BS, CC)

Total Staff Years: .25

Human Research: Human subject research

Keywords: Human Movement

Summary: The ability to accurately predict the effects of disease and treatment on an individual's ability to function relies entirely on our capacity to understand the complex process that transforms muscular effort into functional movements. The purpose of this project was to extend existing human movement analysis methodology by developing analytical techniques that can provide direct estimates of the influence muscular effort has on the movement of all joints, body segments, and overall functional movement task performance. A previous application of one technique to data from a group of normal walkers clearly indicated that the muscles that cross the ankle joint are the primary contributors to normal walking performance. Clinical case studies involving patients with physical impairments continue to reveal a vast array of compensatory movement control strategies. The analytical techniques being developed under this protocol add significantly to the foundation of our ability to ultimately understand the influence of disease on function and to predict the onset of physical disability.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060042-14

LBC: RM

Title: Ultrasound and Videofluoroscopic Imaging in Oral-Pharyngeal Dysphagia in Neurologically Impaired Subjects

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Barbara C. Sonies, Ph.D.

Supervisor of Record: Lynn H. Gerber, M.D.

Collaborators, Lab: Gloria Chi-Fishman, Ph.D. (SLPS, CC)
Jeri L. Miller, Ph.D. (SLPS, CC)

Collaborators, NIH: Marinos C. Dalakas, M.D. (CNP, NINDS)
Mark Hallett, M.D. (NINDS)

Total Staff Years: .8

Human Research: Human subject research: Interviews

Keywords: Swallowing, Neurological Conditions, Dysphagia, Videofluorography, Ultrasound Imaging

Summary: We are currently analyzing the swallowing data from a group of patients with progressive supranuclear palsy who were given denepezil to control neurological symptoms. We designed a study using both ultrasound and videofluorography to examine drug effects on swallowing. Patients were seen for baseline and three follow-up evaluations, in which ultrasound and videofluorographic swallowing studies were administered along with complete oral motor function examinations. Data on swallowing performance in patients with corticobasal degeneration and apraxia of swallowing are still being analyzed. We completed a study to determine the kinematic strategies that are used during randomized discrete and sequential swallows on 30 subjects aged 20 to 79 years. Significant differences were revealed for these two tasks relative to age, gender, and movement of the hyoid bone in support of a theory of motor performance that suggests that the deglutitive motor system is more flexible than previously known. We have completed a study of ten patients evaluating the effects of pallidotomy on swallowing and did not find any significant trends for this procedure on swallowing performance.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060051-05

LBC: RM

Title: Linking Occupational Therapy Process and Patient Performance: The Personal Computer in Occupational Interventions

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan C. Robertson

Supervisor of Record: Lynn H. Gerber, M.D.

Total Staff Years: .1

Human Research: Human subject research

Keywords: Occupational Interventions

Summary: The Occupational Therapy Section has completed a study of occupational therapy process in routine treatment sessions. The purpose of the study was to devise a way to examine process and outcome links in a treatment session. Future plans are to link session outcomes to the overall effect of a treatment program. Twenty patients (ten male, ten female) with a variety of diagnoses (mental illness–35 percent, neurological–25 percent, cancer–20 percent, musculoskeletal–10 percent, and spinal cord injury–10 percent) have participated in the study. Examination of 60 interviews of patients at NIH and the National Rehabilitation Hospital revealed four occupational therapy process variables: occupational form, performance, goals, and reflection. These process variables showed a clear distinction between description (of treatment goals, task, environment, and performance) and analysis, in the form of reflection, during review of experiential learning using typical therapeutic occupations. Descriptive statistics showed that reflection was most frequently cited by patient (48 percent) and therapist (37 to 40 percent) in each of three post-session interviews of patient by treating therapist. Three types of reflection were revealed: content reflection (analysis of occupational form), process reflection (analysis of occupational performance), and premise reflection (analysis of self-management). Further, Spearman Correlation Coefficients found a significant negative correlation between patient performance and reflection for both patient and therapist in all three sessions. Description and analysis are related but separate process variables. Patterns of process in a treatment session are worthy of further examination A follow-up study to compare two interview formats to assess the nature and proportion of reflection in post-session interviews is still under way.

LBC: RM

Title: Rehabilitation Medicine Department Screening Protocol

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Lynn H. Gerber, M.D.

Total Staff Years: .3

Human Research: Human subject research: Minors

Keywords: Locomotion Dysfunction

Summary: The primary function of the Rehabilitation Medicine Department (RMD) is to diagnose and treat patients who have a dysfunction in locomotion, activities of daily living, occupational or avocational roles, communication, deglutition, or chronic pain. The major goal of the department is to help patients achieve maximal function so that they can resume optimal performance in their daily living activities. The screening protocol provides clinicians in the RMD a vehicle for developing and piloting new tests, techniques, technology, and equipment for evaluation and treatment of patients or subjects. The rehabilitation medicine screening protocol was used to pilot the following projects: (1) economics of a manual unilateral eight-muscle subset test compared with 26-muscle test in myositis; (2) feasibility of using Tinnetti assessment in patients with ataxia; (3) study of ULDA to determine feasibility in patients with upper limb impairment; (4) assessment of normal variance of upper extremity girth in premenopausal women; (5) assessment of normal variance in upper extremity girth in postmenopausal women; and (6) evaluation of exercise-induced morphological changes in biceps brachii muscle with digital ultrasound imaging.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060054-01

LBC: RM

Title: Ultrasonic Evaluation of the Development of the Fetal Upper Aerodigestive Tract

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Barbara C Sonies, Ph.D.

Supervisor of Record: Lynn H. Gerber, M.D.

Collaborator, Lab: Jeri L. Miller, Ph.D. (RMD, CC)

Collaborator, Extramural: Christian Macedonia, M.D., Obstetrics and Gynecology
National Naval Medical Center

Total Staff Years: .5

Human Research: Human subject research: Minors

Keywords: Fetal Development, Ultrasound, Swallowing, Respiration, Aerodigestive Tract Development

Summary: We are studying the development of the upper aerodigestive tract, which includes the oropharynx, larynx, pharynx, tongue, and bronchial system, in the fetus, using ultrasound imaging. Pregnant women who receive care at the National Naval Medical Center are randomly selected at their regular ultrasound visits to participate in this study. The regular clinical ultrasound examination is videotaped for later analysis. Both power and color Doppler techniques are used to determine early oral pharyngeal behaviors and to track amniotic fluid flow and vascular sufficiency. Two-dimensional B-mode ultrasound images are obtained to track the growth pattern of the structures of the upper aerodigestive tract. Children who are at risk for developing abnormal feeding at birth will be carefully followed during the course of repeated studies and provided with intervention to facilitate feeding, if required. We hope to develop clinical indicators that signal the possibility of aerodigestive dysfunction after birth. We have evaluated 66 fetuses (GA 15 weeks to 39 weeks) in women aged 19 to 42 and will continue evaluations until we have 100 normal-appearing fetuses and 20 who appear to be at risk for developing delayed feeding patterns.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060055-01

LBC: RM

Title: Task-induced Physiological and Biomechanical Changes of the *In Vivo* Human Tongue

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Gloria Chi-Fishman, Ph.D.

Supervisor of Record: Lynn H. Gerber, M.D.

Collaborators, Lab: Jeri L. Miller, Ph.D. (RMD, CC)
Barbara C. Sonies, Ph.D. (RMD, CC)

Collaborators, NIH: Alan S. Barnett, Ph.D. (NIMH)
John A. Butman, M.D. (CC)
A. Scott Chesnick (NHLBI)

Total Staff Years: .23

Human Research: Human subject research

Keywords: Tongue, *In Vivo*, Human, Volumetrics, Hemodynamics, MRI, Ultrasound, Diffusion Tensor

Summary: The overall objective of this protocol is to acquire a better understanding of the normal physiological responses of the tongue to contraction tasks. Specifically, our goals are (1) to quantify three-dimensional volumetric changes of the tongue as a function of maximal voluntary contraction tasks, (2) to examine task-induced changes in blood flow and how tongue vessels and muscles interact during graded lingual contractions, and (3) to determine task-induced variations in the diffusion properties of water molecules in lingual tissue. The protocol was approved on 12/5/2000. Because of a delay in equipment acquisition and optimization, no studies have been conducted to date. The required equipment includes the following: Sequoia 512 Ultrasound Imaging System (Acuson, CA) and the Digital Swallowing Workstation (Kay Elemetrics, NJ). The ultrasound system is now operational, and optimization of the Digital Swallowing Workstation is expected to be completed by the manufacturer in 2 weeks. We, therefore, project that data collection will begin in October 2001. Despite the delay, we have successfully completed seven Doppler ultrasound pilot studies to refine data collection procedure. In addition, we performed a comprehensive 10-week analysis of the MRI pilot data (six studies) collected from 12/1999 to 8/2000. Preliminary results showed significant tongue volume changes as a function of contraction task. We have quantitative confirmation that our study methods (MRI and ultrasound) are appropriate to address the issues raised in the protocol and will enable us to obtain pioneering information on the functional physiological properties of the *in vivo* human tongue.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060056-01

LBC: RM

Title: Effects of Task on Oral Pressure Dynamics during Swallowing

Dates: from 10/01/2000 to 09/30/2001

Lead Investigator: Gloria Chi-Fishman, Ph.D.

Supervisor of Record: Lynn H. Gerber, M.D.

Collaborators, Lab: Jeri L. Miller, Ph.D. (RMD, CC)
Barbara C. Sonies, Ph.D. (RMD, CC)
Steven J. Stanhope, Ph.D. (RMD, CC)

Collaborator, NIH: Robert A. Wesley, Ph.D. (OD, NCI)

Total Staff Years: .12

Human Research: Human subject research

Keywords: Pressure, Oral, Tongue, Swallowing, Dysphagia, Head-neck Cancer, Myositis, Neurological Disorders

Summary: The overall purpose of this protocol is to characterize normal and abnormal deglutitive tongue pressure as a function of swallowing task. The goals are to: (1) Quantify the normal modulation of propulsive lingual pressure during discrete versus rapid sequential swallows, (2) contrast how task-induced pressure dynamics changes with aging in healthy adults and with reduced tongue strength in neuromuscular and musculoskeletal disorders, (3) determine the relationship between task-induced oral tongue pressure profiles of patients with their diagnostic MBS finding and clinical oral motor signs, and (4) characterize the clinical profiles of patients who can and those who cannot benefit from sequential swallowing as a compensatory strategy. The protocol was approved on 3/28/2001. Because of a delay in equipment acquisition and optimization, no studies have been conducted to date. The required equipment include the following: the Digital Swallowing Workstation (Kay Elemetrics, NJ) and the Digital Fluoroscopy GoldOne System in Diagnostic Radiology. The latter is now operational, and optimization of the Digital Swallowing Workstation is expected to be completed by the manufacturer in 2 weeks. We, therefore, project that data collection will begin in October, 2001. Despite the delay, we have successfully completed four pilot studies, identified the optimal type of Tongue Array (pressure-bulb strip) and adhesive strip to use, and refined procedures for subject screening, task execution, data recording, and data analysis.

TRANSFUSION MEDICINE DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL002040-17

LBC: DTM

Title: Significance of Anti-HIV Antibody in Asymptomatic Donors

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Harvey J. Alter, M.D.

Collaborators, Lab: Cathy C. Conry-Cantilena, M.D. (IDS, CC)
Susan F. Leitman, M.D. (IDS, CC)
Cathy Schechterly, B.A. (IDS, CC)

Total Staff Years: 3

Human Research: Human subject research

Keywords: AIDS, HIV, Blood Donors

Summary: A cohort of anti-human immunodeficiency virus (HIV) positive donors and controls has been under prospective followup since 1985 (*N Engl J Med* 321:917, 1989). At enrollment, 182 subjects were Western blot (WB) positive, including 158 asymptomatic donors, 15 blood recipients, and nine sexual partners. A control population included 70 anti-HIV reactive donors who were WB negative and 21 who were WB indeterminate. Of the 182 WB-positive subjects, 87 percent were donors, 5 percent were sexual partners, and 8 percent were blood recipients. Of the 182 WB positives, 46 (25 percent) are alive and in active followup; 73 (40 percent) are dead, of whom 62 (85 percent) died of AIDS; 63 (35 percent) are lost to followup (LTFU); 13 of the 73 LTFU were known to have AIDS at the time they left the study. Of the 46 in active followup, 77 percent are male and 91 percent were detected at blood donation. Of the 46 active patients, 17 (37 percent) have had an AIDS-defining event. Others have CD4 counts under 300 but have had a stable course even before treatment. A subset of 13 patients has exceeded 10 years of followup; they have CD4 counts persistently more than 400 with no AIDS-defining infections and no physical abnormalities except minor adenopathy. Our goal will be to focus on this group in terms of predictive factors for long-term nonprogression. We are in the process of measuring serial viral loads in the entire cohort dating back to 1985 and will compare these to CD4 counts and outcome. HIV co-receptors will be measured as indicated. No evidence of HIV infection evolved in the 91 subjects who were initially anti-HIV positive, but WB-indeterminate or WB-negative. Treatment with HAART therapy is being conducted by personal physicians or through other NIH protocols.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002045-15

LBC: DTM

Title: Etiology of Allergic Reactions in Platelet and Granulocytapheresis Donors

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborator, Extramural: Kearby Fugate, Ph.D.
CDRH, FDA

Total Staff Years: .1

Human Research: Human cells or tissues

Keywords: Allergic Reactions, Platelet and Granulocytapheresis Donors

Summary: In February 1984, the DTM converted from manual to automated platelet collection techniques. During the next 10 years, 26 donors undergoing apheresis procedures on the Fenwal CS-3000 device experienced acute hypersensitivity reactions. Sixteen reactions occurred during plateletpheresis and ten reactions occurred during granulocytapheresis procedures. Using a combination of skin tests, radioallergosorbent tests (RAST), and basophil histamine release assays, specific IgE-mediated sensitization to ethylene oxide—a gas used to sterilize the plastic disposable apheresis kits—was found in ten of 16 plateletpheresis donors and eight of ten granulocytapheresis donors experiencing reactions but in none of the 140 nonreacting controls. Donors with documented EO sensitization were permanently deferred from subsequent apheresis donations. The results of these studies were reported to the manufacturer of the CS-3000 apheresis device and to FDA. As a result of these reports, the manufacturer of the CS-3000 disposable apheresis kits changed its sterilization techniques from predominantly EO exposure to predominantly gamma irradiation. Since this change, there have been only two documented cases of EO hypersensitivity reactions in DTM donors, in August 1995 and March 1998. However, in August 1997, a donor in another blood center had an acute fatal anaphylactic reaction during plateletpheresis and was found on postmortem testing to have high titer IgE anti-EO. This was the first report of a lethal allergic reaction to EO in an apheresis donor and has reopened the question of prospective screening of all apheresis donors for EO sensitization. We have estimated that as many as 1 percent of all repeat apheresis donors may become sensitized to EO, although only a fraction of those who are sensitized will have clinically evident allergic reactions. To document the current EO sensitization rate among donors and to compare this rate with individuals who have occupational exposure to EO, we have established a collaborative effort with CBER/FDA. Screening of approximately 500 healthy repeat apheresis donors using both an established RAST test and an experimental enzyme immunoassay (EAI) test for IgE anti-EO will be performed. A cohort of serum samples stored at the Centers for Disease Control and Prevention and derived from individuals with allergic reactions presumed to be due to EO sensitivity will also be tested, as will a large group of samples derived from the National Health and Nutritional Examination Survey study.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002055-13

LBC: DTM

Title: Kinetic Studies of Indium-labeled Leukocytes

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborator, NIH: James Yang, M.D., Ph.D. (SB, NCI)

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Kinetic Studies, Indium-labeled Leukocytes

Summary: The kinetic patterns of fresh, frozen-thawed, or cultured human leukocytes are studied by tagging the cells *ex vivo* with 111-indium, a radioisotopic label, and measuring their distribution throughout the body by means of gamma camera imaging and gamma counting of blood samples. The most common use of radiolabeled leukocyte trafficking studies is for abscess localization in cases of suspected infection not definitively diagnosed by other noninvasive studies. In these cases, autologous or allogeneic granulocytes, collected by simple phlebotomy or by apheresis, are labeled with 50 uCi of 111-indium per kg of patient weight, not to exceed 500 uCi total. Twenty-four studies for the purpose of localizing abscesses were performed in Clinical Center patients enrolled in a variety of protocols over the past 9 years. Of these 24, three were positive and received appropriate therapy: a hemophiliac patient with HIV infection was treated for salmonella osteomyelitis, a patient with aplastic anemia was treated for staphylococcal pneumonia, and a patient with Zollinger-Ellison syndrome was treated for diverticular abscess. In many of the negative studies, unnecessary surgical exploration was avoided. Three of the 24 indium-labeled allogeneic leukocyte studies were performed in patients with chronic granlomatous disease: in the first case to document clearance of pulmonary infection, in the second to confirm lack of trafficking in an alloimmunized recipient, and in the third to localize a cause for fever. In all three cases, gamma imaging results were used to guide therapy, specifically, to terminate a course of granulocyte transfusions when they were either no longer necessary or were ineffective or to prevent an exploratory laparotomy. Collaborative trials with the Surgery Branch, NCI, have investigated the diagnostic utility and prognostic application of radiolabeled autologous tumor infiltrating lymphocyte (TIL) studies in patients with metastatic melanoma. TIL trafficking studies revealed metastatic deposits that were undetected clinically, and TIL trafficking to sites of tumor was strongly correlated with tumor regression following TIL infusion. In contrast, studies of indium-labeled autologous cloned T cells with antimelanoma activity did not show any trafficking to tumor sites in 14 patients. The results of the trafficking studies were highly predictive of clinical response, in that none of the 14 patients had regression of disease in response to the cloned anti-melanoma T cells. On the basis of these studies, the protocol was terminated, and a modified approach, using highly immunosuppressive conditioning prior to infusion of autologous cloned T cells, will be tested. Trafficking of indium-labeled autologous cells will be used as one of the benchmarks with which to evaluate the response to therapy.

LBC: DTM

Title: Treatment of Familial Hypercholesterolemia by Dextran Sulfate Adsorption Apheresis

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborator, NIH: Robert D. Shamburek, M.D. (MDB, NHLBI)

Collaborator, Extramural: Deno Zachary, Sc.B.
Kaneka America Corporation

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Hypercholesterolemia

Summary: Patients with familial hypercholesterolemia (FH) type IIa are at high risk of premature coronary artery disease due to elevated low density lipoprotein (LDL) and Lp(a) cholesterol levels. Diet and drug therapy can reduce cholesterol concentrations in most patients with heterozygous FH, but a small proportion of heterozygotes and nearly all homozygotes do not respond to therapy. Selective removal of LDL by dextran sulfate affinity adsorption was evaluated in these patients in a collaborative multicenter U.S. study. The dextran sulfate apheresis system (Liposorber LA-15, Kaneka, Japan) removed LDL and Lp(a) without lowering HDL or albumin levels, thus avoiding the need for colloid replacement solutions. Six FH patients were enrolled at the Clinical Center; the total cohort enrolled nationwide included ten homozygotes and 54 heterozygotes. Treatments were administered at 7 to 14 day intervals. Mean acute reductions in total, LDL, and Lp(a) cholesterol levels were 70, 81, and 68 percent, respectively, in homozygotes and 61, 76, and 65 percent, respectively, in heterozygotes. The treatments were very well tolerated. The results of the multicenter study suggest that dextran sulfate adsorption is a safe and effective way to clear plasma of LDL cholesterol and has the advantage, compared with simple plasma exchange, of eliminating the need for colloid replacement solutions. The data gathered in this study were used as the basis for licensure of the LA-15 system, which was approved by the FDA for treatment of FH in July 1996. Patients are now continuing long-term followup on an LDL-apheresis registry to gather post-licensure data on the effect of long-term treatment on development of primary and secondary atherosclerotic events and on overall survival. A 5-year interim analysis of 49 of the original 64 patients who received long-term LDL-apheresis was performed. There was a 44 percent reduction in cardiovascular events during the 5 years the patients received LDL-apheresis compared with the 5-year period prior to LDL apheresis (3.5 events per 1,000 patient-months of treatment compared with 6.3 events per 1,000 patient-months before LDL-apheresis therapy). These findings support the long-term safety and clinical efficacy of LDL apheresis in patients with FH who are inadequately controlled with drug therapy. Two patients are continuing to receive regular biweekly LDL apheresis treatments at the Clinical Center, while a third patient, a child, is receiving biweekly plasma exchange until his blood volume becomes large enough to tolerate the extracorporeal volume of the LDL apheresis device.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002064-10

LBC: DTM

Title: Characterization of Newly Identified Viral Genomes and Their Clinical Correlations

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James Waikuo Shih, Ph.D.

Collaborators, Lab: Harvey J. Alter, M.D. (DTM, CC)
Richard Y. Wang (DTM, CC)

Total Staff Years: 1.5

Human Research: Human cells or tissues

Keywords: Viral Genomes

Summary: There are two components in this project, and both are extended from our continuous commitment to the clinical investigation of viral hepatitis. One of these is an effort to respond to the increasing demand for a more precise measurement of relevant genomic information in any viral infection. The knowledge of the presence of a specific viral gene will help in identifying the infectious agent. However, to assess the stage of a disease, to evaluate the efficacy of a treatment, to determine the value of a predictor in the progression of a disease, and to monitor the patient's disease progression, a more precise and quantitative analysis of the specific gene would be required. This previous research-oriented question can now begin to be answered in routine clinical laboratories with the advanced technology of molecular biology, such as polymerase chain reaction (PCR), and sequencing and mapping of the restriction nuclease digested fragments. We initiated developmental research in molecular diagnostic technology to meet our clinical study need for HBV, HCV, and HIV infection. Whenever possible, we would improve the basic PCR technique to become a semi-quantitative procedure. During the last 2 years, we were able to apply the same principles of using PCR as primary study tool for viral infection to several newly identified human hepatitis viruses or suspected hepatitis viruses such as HGV, TTV, and SENV. We found that these viruses were indeed transmissible by blood transfusion but have little or no impact on post-transfusion hepatitis. Although specific HGV RNA was identified in both recipients and paired donors sera, it could also be found in non-transfused controls. It could be found in patients with chronic infection with mild or no observed liver function abnormality, but the causative relationship could not be determined. The prevalence of these viruses in blood donors, in general, was higher than that of HCV. The other part of this project is related to viral discovery. We have always maintained an effort to find other causative viral agents that may be responsible for hepatitis cases with unidentifiable cause. Because of its great resource requirement, we tried to conduct this project with industry partners under CRADA. We divided responsibilities by concentrating our group in confirming initial discovery and clinical characterization. In the past few years, we also engaged in developing cloning techniques for rare event genes that might identify low copy infectious agents from patient sera or tissues. The techniques developed were unique and had the potential to be applied to a large number of specimens at the same time. An invention report has been filed with the NIH Technology Transfer Office and is being considered for possible patent application.

LBC: DTM

Title: A Prospective Study of Anti-Hepatitis C Virus-positive Blood Donors

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Harvey J. Alter, M.D.

C*ollaborators, Lab:* Cathy C. Conry-Cantilena, M.D. (IDS, CC)
Susan F. Leitman, M.D. (IDS, CC)
Cathy Schechterly, B.A. (IDS, CC)
James Waikuo Shih, Ph.D. (IDS, CC)

Collaborator, NIH: Jay Hoofnagle, M.D. (DDN, NIDDK)

Collaborators, Extramural: Joan Gibble, M.D., American Red Cross (Chesapeake)
Paul Ness, M.D., Department of Lab Medicine, Johns Hopkins University

Total Staff Years: 3

Human Research: Human subject research

Keywords: Hepatitis C Virus, HCV, Hepatitis C, Blood Donor, RIBA, Anti-HCV, HCV RNA

Summary: This protocol is designed to study the natural history and epidemiology of hepatitis C virus (HCV) infection in an asymptomatic blood donor population. Thus far, 720 subjects have been enrolled, including 422 recombinant immunoblot assay (RIBA) positives, 186 RIBA indeterminates, and 112 RIBA-negative controls. The early data have been published (*New England Journal of Medicine* 334:1691,1996), and the trends have remained the same over time. Unexpected findings were the high proportion (41 percent) of RIBA-positive donors who admitted to prior (remote) intravenous drug use and the strong independent association between cocaine snorting, and HCV positivity. Shared paraphernalia for snorting accompanied by epistaxis, may serve as a covert vehicle for parenteral viral transmission. Among anti-HCV+/RIBA-positive donors, 87 percent were persistently viremic, but 13 percent appeared to have recovered from prior HCV infection. A liver biopsy has been obtained from 135 patients who were chronically infected; 51 percent had mild chronic hepatitis and 44 percent had moderate chronic hepatitis. Despite a mean duration of infection of 20 years, only 5 percent had severe inflammation, 10 percent significant fibrosis, and 1.5 percent cirrhosis. Overall, HCV infection in this cohort was generally asymptomatic and clinically benign. Despite an association of HCV with sexually promiscuous practices, we found no evidence for sexual transmission to the specific partners of 116 HCV-infected individuals. The study continues to follow the natural history of HCV infection and is now focusing on histologic progression as assessed in liver biopsies obtained at 5-year intervals. New emphasis is being placed on studies of cell-mediated immune responses to HCV and of treatment responses.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002071-06

LBC: DTM

Title: Dissecting the Molecular Immunology of T Cell-aimed Vaccines

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: David Frank Stroncek, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborator, NIH: Franco Marincola, M.D. (CC)

Total Staff Years: 5

Human Research: Human subject research

Keywords: Human Leukocyte Antigen, HLA, Immunogenetics, Vaccination, TCR, Polymorphism

Summary: Human leukocyte antigen (HLA) genes are the most polymorphic genes in the human genome. Knowledge about HLA polymorphism in relation to possible peptide-based, T cell-restricted vaccination protocols is important for understanding the physiology of T-cell recognition and to improve strategies of T cell antigen-specific vaccination. During the last few years, the HLA Laboratory has developed and perfected techniques for high-resolution typing of HLA class I and class II molecules using polymerase chain reaction (PCR) techniques and, more recently, robotic sequencing. With these high-resolution methods it has been possible to achieve several categories of results: (1) Development of an antigen identification program based on co-transfection of HLA and cDNA libraries into permissive antigen-presenting cells (in this fashion, a new epitope for anticancer treatment was identified this year in our lab). (2) Development of a technology for the preparation of epitope/HLA tetrameric complexes for various HLA alleles and various minimal epitopic sequences that can be used for patient monitoring during vaccination as well as sorting of relevant antigen-specific T cells. (3) Preparation of a cDNA library of various HLA alleles to be readily available to our lab and the community at large for the previously mentioned purposes. (4) Development of high-sensitivity and high-throughput technology for the *in situ* monitoring of T-cell responses during vaccination against cancer, by measuring serial gene expression levels using quantitative real-time PCR and cDNA microarray technology. With these techniques we are actively investigating variables involved in the algorithm-modulating tumor/host interactions in the context of active vaccination protocols.

LBC: DTM

Title: Evaluation of Nucleic Acid Vaccine as a Preventive and Therapeutic Modality

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James Waikuo Shih, Ph.D.

Collaborators, Lab: Xuanmao Jiao, Ph.D. (DTM, CC)
Richard Y. Wang (DTM, CC)

Total Staff Years: 2.8

Human Research: Neither human cells nor tissues

Keywords: Nucleic Acid Vaccine

Summary: This program is extended from our continuing efforts to investigate the immune response to the hepatitis C virus (HCV) in both humans and experimental animals. In extensive earlier studies, we identified immunodominant and neutralizing epitopes on the hepatitis C virus that will now be further investigated to examine the relationship between immune response and persistent infection. In FY95 and FY96, we initiated a new project to examine the potential of nucleic acid vaccination for the prevention and/or treatment of HCV infection. The long-term goal of both the basic immunology studies and the DNA vaccine studies is to develop models for immune therapy for chronic viral infections of the liver. One of the advantages of genetic immunization is that the endogenously expressed proteins can be recognized by class I MHC molecules and expressed on the cell surface. The MHC-antigen complex on the cell surface can be recognized by cytotoxic T lymphocytes (CTL), which, in turn, are activated and attack infected cells. The possibility of inducing an immune response to HCV core protein using DNA immunization provides an attractive alternative to classic vaccination. There are many problematic issues related to the vaccine development for hepatitis C. One major concern is the genetic instability of the infectious agent. There are two hypervariable regions in the putative HCV envelope proteins. Immune escape mutants have been attributed to mutations in these regions. Experimentally infected chimpanzees and HCV-infected patients have been found to repeat bouts of infection with either homologous or new strains of HCV. This failure to develop protective immunity links to the high chronicity rate in HCV infection. Directly inducing strong cell-mediated immunity, especially protective cytotoxic T lymphocyte responses, may not only help in preventing initial HCV infection but may serve as a mechanism for immune modulation to overcome existing infection. Using the mouse model, we were able to evaluate the induction of antibodies to several different plasmid constructs containing both HCV structural and nonstructural genes. We were also able to develop assays to measure both humoral and cell-mediated immune responses, including CTL activities, in the mouse model. In the past year, we have tested the genetic sequences of many HCV-related immunogens to establish the best candidate DNA vaccine. We have also studied methods of vaccine delivery and immunity augmentation procedures; accumulated extensive experience in measuring humoral and cell-mediated immunity; and developed effective immunization strategies in small experimental animals. We believe we are now ready to test our findings in the only animal model susceptible to HCV infection, the chimpanzee. Protocols are being written for DNA vaccination in the chimpanzee using constructs containing genes for HCV core and envelope proteins.

LBC: DTM

Title: Viral and Immune Factors that Influence Recovery or Progression of Hepatitis C

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Harvey J. Alter, M.D.

Collaborators, Lab: Cathy Schechterly, B.A. (IDS, CC)
James Waikuo Shih, Ph.D. (IDS, CC)

Collaborators, NIH: Robert Purcell, M.D. (LID, NIAID)
Barbara Rehermann, M.D. (LD, DDB, NIDDK)

Collaborator, Extramural: Patrizia Farci, M.D., Department of Medicine, University of Calgiari

Total Staff Years: 1.3

Human Research: Human subject research

Keywords: Hepatitis C, Viral and Immune Factors

Summary: Approximately 15 percent of patients recover from hepatitis C virus (HCV) infection while 85 percent become persistently infected with various degrees of associated chronic liver disease. In this study, comparisons will be made between patients who recover rapidly, those who have delayed recovery, those with persistent infection and stable chronic disease, and those with rapidly progressive, fatal infection. The parameters measured will include viral burden (initially and over time), HCV genotype, the number of viral quasi-species (extent of viral heterogeneity) at the time of infection and subsequently, neutralizing antibody responses, and, if appropriate technology is available, cytotoxic T-cell responses. The goal is to determine if any of these parameters can predict outcome. Studies to date have shown no correlation with genotype, as the population is fairly homogeneous for HCV genotype 1. However, there does appear to be a correlation between viral quasi-species and disease outcome. Using rare specimens obtained during the first 16 weeks of HCV infection, we have measured the mean Hamming distance that reflects the extent of viral diversity (the degree of sequence divergence within the viral quasi-species). We have found that the mean Hamming distance 12 to 16 weeks after the onset of acute infection predicts whether the patient will recover from HCV infection or develop persistent infection and chronic liver disease. Patients who recover have a declining Hamming distance as antibody to HCV develops, signifying immunologic containment and then clearance of the virus. In contrast, the majority of patients demonstrate an increased mean Hamming distance as antibody appears. This suggests that if the immune response is not sufficient to clear the virus, it paradoxically exerts immune pressure that results in mutations (escape variants) that lead to persistent infection. Interestingly, patients with fulminant hepatitis have a very low degree of viral diversity because they succumb to the infection before the immune system can clear the virus or exert immune pressure. This study has been published (*Science* 288:339-344, 2000). In the next phase of this study, we are going to measure the quasi-species throughout the long-term course of HCV infection and the relation of the quasi-species to treatment responses. In addition, we will identify patients with newly acquired acute hepatitis C so that we can serially measure viral load, viral quasi-species, neutralizing antibody responses, and, particularly, cell-mediated immune responses. Because such infections are no longer occurring in the transfusion setting, we will follow commercial plasma donors and health care workers who sustain needle-stick injuries.

LBC: DTM

Title: Hepatitis C Virus Infection in Infants and Children

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Harvey J. Alter, M.D.

Collaborator, Lab: Mary Helen Boone (IDS, CC)

Collaborators, Extramural: Camilla Baxter, B.S., Hematology, Childrens National Medical Center
Naomi Luban, M.D., Hematology, Childrens National Medical Center
Parvathi Mohan, M.D., Gastroenterology, Childrens National Medical Center

Total Staff Years: .2

Human Research: Human subject research: Minors

Keywords: Hepatitis C, Hepatitis C in Infants and Children

Summary: It has become apparent from multiple studies that hepatitis C virus (HCV) infection is very indolent and that serious sequelae (cirrhosis, carcinoma) occur in less than 15 percent of persons during their first 20 years of infection. It is presumed that the proportion with severe outcomes will increase as the duration of followup increases, and it may be that those infected at a young age will fare worse because they have 3 to 8 decades for HCV infection to evolve into overt liver disease. This study, conducted in collaboration with Children's National Medical Center (CNMC), has identified infants and children who were transfused at CNMC from 1983 to 1992, the decade just prior to second generation anti-HCV testing. During this interval, 5,546 children who met eligibility criteria were transfused at CNMC. The mean age at transfusion was 1 year (range: birth to 10.7 years). Thus far, 2,668 children (49 percent) have been recalled and provided consent/assent. The mean age at testing was 11 years (range: 4 to 17 years). Of the 1,753 children fully tested for antibodies to HCV and hepatitis G virus (HGV), 36 (2.0 percent) are anti-HCV positive and 100 (5.7 percent) are HGV RNA positive. The HCV and HGV prevalence in age-matched non-transfused controls are 0.3 and 6.3 percent, respectively. There is a significant association between HCV infection and transfusion, but the overall prevalence is lower than expected given that these children were transfused prior to HCV donor screening. The 36 HCV-infected children have been followed for a mean of 24 months. All are asymptomatic. The range of ALT is 29 to 140 IU/ml; 80 percent have at least one ALT value that exceeds 1.5 times the upper limit of normal. In an adjunctive study, liver biopsies have been performed on 25 children, 16 of whom are included in this transfusion look-back study. The average interval from transfusion to biopsy was 10.7 years. The histologic lesions were generally mild, but four (16 percent) had bridging fibrosis. None had cirrhosis. Duration of infection and age at infection did not appear to influence the extent of fibrosis. In the final analysis, this study will determine the minimal rate of transfusion-transmitted HCV and HGV infection in the decade before anti-HCV testing and will allow for an annualized incidence estimate and a determination of the national burden of transfusion-induced viral hepatitis in children. To date it appears that persistent infection and chronic liver disease are less common in children than adults, but continued long-term followup with serial liver biopsies is necessary before the true disease burden can be ascertained. This study will have major implications for antiviral therapy programs and might serve to shift emphasis to pediatric populations, where response rates may be higher and the long-term benefit greater.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002080-06

LBC: DTM

Title: Natural History of Hepatitis C Virus Infection

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Harvey J. Alter, M.D.

Collaborator, NIH: Leonard Seeff (DDP, NIDDK)

Total Staff Years: .5

Human Research: Human subject research

Keywords: Hepatitis C, Natural History

Summary: Patients enrolled in NIH prospective studies of transfusion-associated hepatitis have been followed long term to determine the persistence of hepatitis C virus (HCV) infection and the chronic consequences of that infection. Eighty-five percent of patients infected with HCV became chronic carriers, and 15 percent resolved their infection, usually within 1 year of onset. The vast majority of patients with persistent viremia have some evidence of chronic hepatitis based on serial alanine aminotransferase (ALT) determinations and liver biopsy. Of those biopsied, approximately 20 percent have histologic evidence of cirrhosis, though only half of those patients have had clinical evidence of cirrhosis. Liver-related mortality within the first 2 decades of followup has been 4 percent. These NIH patients were incorporated into a multi-center study of 568 persons with transfusion-associated non-A, non-B hepatitis (predominantly hepatitis C), and 984 matched controls who were transfused but did not develop hepatitis. After an average followup of 18 years, all cause mortality was 51 percent in the hepatitis group and 52 percent in the controls (NS). There was a slight increase in liver-related mortality in the hepatitis group (3.3 percent vs. 1.4 percent, $p = .03$). Seventy-one percent of the deaths due to liver disease occurred in patients with associated chronic alcoholism. Twenty-year morbidity followup of 103 HCV-positive individuals shows that 77 percent have persistent infection, 17 percent have recovered but maintain antibody to HCV, and 6 percent show no serologic or molecular evidence of their prior HCV infection. Less than 15 percent have developed cirrhosis; in the absence of cirrhosis, there is virtually no clinical evidence of this longstanding HCV infection.

LBC: DTM

Title: Studies of Viral Hepatitis and AIDS in the Chimpanzee Model

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Harvey J. Alter, M.D.

Collaborator, Lab: Mary Helen Boone (IDS, CC)

Collaborator, NIH: Barbara Rehermann, M.D. (LD, DDB, NIDDK)

Collaborators, Extramural: Michael Busch, M.D., Ph.D., Department Transfusion Medicine
Blood Centers of the Pacific
Krishna Murthy, D.V.M., Virology/Immunology
Southwest Foundation for Biomedical Research

Total Staff Years: .1

Human Research: Human subject research

Keywords: Viral Hepatitis, AIDS

Summary: This laboratory, in collaboration with the Southwest Foundation for Biomedical Research in San Antonio, TX, has performed a series of studies in the chimpanzee model, including the initial transmission of the non-A, non-B hepatitis agent that subsequently proved to be the hepatitis C virus. Current studies in this model include the following: (1) We have previously used the chimp model to define the early events of HIV infection and had evidence from serial transmission studies that blood did not transmit HIV during the incubation period of the infection prior to the first detection of HIV RNA. This evidence suggests that molecular assays for HIV that were introduced into blood screening might totally abrogate the infectious window and prevent blood transmission of HIV. Similar studies are now being performed for hepatitis C virus (HCV) infection to determine if nucleic acid testing (NAT) of donors could completely block HCV transmission. (2) *Viral Inactivation*: In collaboration with Cerus Corp., the chimp model was used to establish the efficacy of psoralen/UV-inactivated platelets. This is the first viral inactivation procedure that maintains the integrity of the cellular components of blood. Three chimpanzees have each been exposed to infectious doses of HCV and hepatitis B virus (HBV) that have been psoralen-UV treated. After 1 year of followup, no animal was infected with either HBV or HCV. This study is now being repeated with psoralen/UV-inactivated plasma. These animal studies confirm *in vitro* efficacy data and set the stage for safety and efficacy trials in humans. This method should have broad application for platelet transfusion therapy and, ultimately, for plasma and red cell transfusion as well.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002084-06

LBC: DTM

Title: Development of Methods for *Ex Vivo* Cultured and Immunologically and/or Genetically Modified Cells

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Elizabeth J. Read, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborators, NIH: A. John Barrett, M.D. (NHLBI)
Daniel H. Fowler (MB, NCI)
Ronald E. Gress, M.D. (EIB, NCI)
Harry L. Malech, M.D. (LHD, NIAID)
Scott Solomon, M.D. (HB, NHLBI)

Total Staff Years: 1

Human Research: Human subject research: cells or tissues

Keywords: *Ex Vivo*, Cultured, Genetically Modified Cells

Summary: Preclinical development of complex processing systems for *ex vivo* culture-expanded lymphohematopoietic cells, with subsequent immunologic and/or genetic manipulation, have been carried out in collaboration with a number of NIH institute investigators. (1) Preparation of allogeneic donor lymphocytes selectively depleted for alloreactive T-cells using an anti-CD25 immunotoxin (collaboration with NHLBI, Bench-to-bedside award): During this year, we completed development and scale up of this complex process, and in September 2001 initiated a phase I clinical trial of selectively depleted donor-specific alloreactivity in the setting of allogeneic hematopoietic transplantation. (2) Preparation of donor Th2 T-cells for clinical trials (collaboration with NCI): Development of this process incorporated CD8/CD20 depletion and CD3/CD28 bead stimulation, which produces a lymphocyte product that is 95 percent CD4+ and <1 percent CD8+. The clinical trial was initiated in March 2001, and nine patients have been treated to date (three on dose level I, six on dose level II). Dose level III patients will be treated in early FY2002. (3) Fibronectin transduction: A method for improved gene transduction using fibronectin-coated bags was previously developed and incorporated into the clinical trial of gene therapy for chronic granulomatous disease. At the end of FY2001, this method was adapted to a new clinical trial of gene therapy in ADA deficiency that takes advantage of new vectors. To date, very high transduction efficiencies (80 percent) have been observed.

LBC: DTM

Title: Methods for Positive and Negative Selection of Hematopoietic Progenitor Cells

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Elizabeth J. Read, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborator, Lab: Charles S. Carter (CELL, CC)

Collaborators, NIH: Austin John Barrett, M.D. (NHLBI)
Ronald E. Gress, M.D. (EIB, NCI)
Harry L. Malech, M.D. (LHD, NIAID)

Total Staff Years: 1.5

Human Research: Human subject research: cells or tissues

Keywords: Hematopoietic Progenitor Cells

Summary: Preclinical and clinical studies of automated closed systems for positive and negative selection of lymphohematopoietic cells have been done in collaboration with biotechnology firms, which have developed systems for potential application to clinical cellular therapies: (1) CellPro T-Cell Depletion System: A clinical evaluation of this two-step positive (CD34) and negative (CD2) selection system, which uses an immunoabsorption approach, was completed in August 1998. This study randomized 24 allogeneic donors to fresh versus pooled processing of stem-cell apheresis products. Results demonstrated equivalence between the two study arms in processing and clinical outcomes, so the pooled processing approach was used for practical and economic reasons (less processing time, lower costs associated with use of one expensive system vs. two). This system is no longer clinically available. A manuscript comparing results of this system with the Nexell Isolex system was published in October 2001 (Nakamura et al. *Br J Haematol*). (2) Nexell, Inc. Isolex: Studies of the automated Isolex 300i for immunomagnetic selection of hematopoietic progenitor cells were completed. Over 100 selection procedures on version 2.0 (either positive only or combined positive/negative selection) have been completed, and over 100 selection procedures on version 2.5 were completed over the past 3 years. Studies of combined positive/negative selection aimed to achieve maximum T-cell depletion of peripheral blood stem-cell products have led to incorporation of this method into several allogeneic transplantation protocols. Results on the version 2.5 combined positive/negative procedure show a mean CD3+ T-cell depletion of 5 logs, with mean CD34+ cell recovery of 60 percent. Evaluation of different T-cell antibodies (CD2 alone vs. CD4+CD8 vs. CD2+CD6+CD7) demonstrated equivalence in the combined positive/negative method. This method will continue to be used in clinical trials. (3) Miltenyi CliniMacs: In FY2000, we performed a preclinical study of positive selection of normal donor-mobilized PBSC using this system. Results show a mean CD34+ cell recovery of 55 percent and a mean CD3+ cell depletion of 5 logs. This system may be incorporated into future clinical trials.

LBC: DTM

Title: Therapeutic Efficacy of Granulocyte Colony-Stimulating Factor-Mobilized Granulocyte Concentrates

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

S*upervisor of Record:* Harvey Klein, M.D.

Collaborator, Extramural: Jaime Oblitas, M.T., Department of Transfusion Medicine, NIH

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Granulocyte Transfusions, Granulocyte Colony-stimulating Factor

Summary: The efficacy of therapeutic granulocyte transfusions is limited by the relatively small number of cells obtained using standard apheresis techniques. In prior studies, we demonstrated that granulocyte concentrates prepared by granulocyte colony-stimulating factor (G-CSF) or the combination of G-CSF and dexamethasone (dexa) stimulation of the donor contained 2.3- and 3.5-fold greater numbers of granulocytes than products prepared using dexamethasone alone (product content 2.09 x 10^{10} cells with dexamethasone alone vs. 4.87 and 7.31 x 10^{10} cells total with G-CSF and G-CSF plus dexa, respectively) ($p < 0.01$ for dexa vs. G-CSF alone or G-CSF plus dexa). Seventy-two percent of donors getting G-CSF plus dexa experienced restlessness, insomnia, bone pain, or headache. Ten percent of donors requested discontinuation of participation in the study because of the inconvenience and discomfort of the mobilization regimen. Forty-nine Clinical Center patients have received G-CSF mobilized granulocytes. Thirty were profoundly neutropenic, including 13 patients with severe aplastic anemia (SAA), ten stem cell transplant recipients, six patients with lymphoma, and one with breast cancer. The remaining 19 patients had CGD. In the neutropenic patients, 19 had systemic filamentous fungal infections, nine had bacterial infections, and one each had RSV or candidemia. The mean increment in granulocyte count 1 hour post-transfusion was 2600/uL, and counts greater than 500/uL above baseline were sustained for 12 to 24 hours. One of the 11 neutropenic, immunosuppressed patients who survived longer than 2 weeks after the initiation of granulocyte transfusions developed HLA allosensitization, as did two of the 13 CGD patients. In the absence of HLA allosensitization, granulocyte transfusions were associated with progressive hypoxia, pulmonary infiltrates, and an ARDS-like event in four of 13 SAA patients, vs. one of 19 CGD patients. Of the neutropenic patients with tissue molds, nine of 19 stabilized or improved during granulocyte transfusion therapy, but only four of 19 survived hospitalization. In contrast, three of nine with bacterial processes were discharged from the hospital. Sixteen of 19 patients with CGD had complete resolution of their fungal (eight of 11) or bacterial (eight of eight) infections. These pilot studies of G-CSF-mobilized granulocytes suggest that they may confer survival benefit in carefully selected neutropenic patients with life-threatening infections but may be associated with significant progressive pulmonary toxicity. A randomized prospective study of the efficacy of G-CSF-mobilized granulocyte transfusions in patients with severe aplastic anemia, hospitalized at the Clinical Center, is being considered to further delineate the benefit to risk profile of this therapy.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002091-05

LBC: DTM

Title: Facilitation of Peripheral Blood Stem Cell Transplants by National Marrow Donor Program

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborators, Extramural: Robyn Ashton, R.N., Marrow Donor Center, DTM, NIH
Dennis Confer, M.D., Chief Medical Officer, NMDP

Total Staff Years: 1.2

Human Research: Human subject research

Keywords: National Marrow Donor Program, Peripheral Blood Stem Cell Transplants

Summary: The National Marrow Donor Program (NMDP) was established in 1987 to (1) create a registry of volunteer, tissue-typed, unrelated bone marrow donors and (2) facilitate matched unrelated donor marrow transplants through a coordinated circuit of donor, collection, and transplant centers. As of July 31, 2001, 4.46 million donors were participating in the registry and 12,748 unrelated stem cell transplants had been facilitated. Peripheral blood stem cell (PBSC) components, harvested by apheresis of filgrastim-stimulated donors, provide larger numbers of progenitor cells, which engraft more rapidly than marrow-derived cells and are being increasingly used instead of marrow in both the related-and unrelated-donor settings. The NIH Marrow Donor Center, one of the largest hospital-based donor centers participating in the NMDP network, with 60,000 donors on its registry, is participating in two nationwide NMDP protocols: one for acquisition of filgrastim-stimulated PBSCs for primary unrelated donor transplant and one for acquisition of PBSCs for second transplants (necessitated by rejection or tumor recurrence after a first transplant). The objectives of these studies are (1) to monitor the safety of filgrastim administration in healthy volunteer donors, (2) to compare the adverse effects of bone marrow vs. PBSC donation, and (3) to monitor the outcome of matched unrelated-donor PBSC transplants, including time to engraftment, incidence of GVHD, and disease-free and overall survival. As of September 30, 2001, 41 NIH donors had undergone 55 apheresis procedures to collect PBSCs for unrelated NMDP recipients. Twenty-seven of 41 (66 percent) required only a single apheresis procedure to collect an adequate cell dose for transplant. Fourteen of 41 had a poor CD34 mobilization response to filgrastim and needed two consecutive apheresis procedures to collect an adequate cell dose. One of the 41 required a central line. All donors experienced G-CSF-induced fatigue, insomnia, bone pain, or headache, although these effects were considered severe in only 8 percent. Peak mean leukocyte counts after filgrastim were 45,000/uL, and post-apheresis thrombocytopenia (less than 100,000/uL) occurred in two of 13 donors (15 percent), both of whom underwent two procedures. The mean time to complete recovery from PBSC donation was 1 week, compared with 3 weeks for marrow harvest. Eight of 13 donors preferred G-CSF-stimulated apheresis donations to marrow harvest because of the lack of need for anesthesia and hospitalization; the discomfort of the two procedures was considered equivalent. Recipient outcomes—including time to engraftment, GVHD incidence and severity, and overall survival—have not yet been evaluated. Administrative and statistical support for this study are provided by the NMDP National Office. Filgrastim is provided under an IND agreement with Amgen (BB-IND #6821).

LBC: DTM

Title: Structure and Function of Granulocyte Antigens

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: David Frank Stroncek, M.D.

Supervisor of Record: Harvey Klein, M.D.

Total Staff Years: .5

Human Research: Human subject research

Keywords: Granulocyte Antigens

S*ummary:* Granulocyte antigens play an important role in cell functions including adhesion, cell activation, and binding of immunoglobulins. The purpose of these studies is to better define the molecular basis of variations in neutrophil antigens and their role in neutrophil function. The Fc-gamma-receptor IIIb (Fc-gamma-RIIIB) genes that encode neutrophil-specific antigens NA1 and NA2 differ at five nucleotides, four that result in amino acid differences among the two alleles. We have described people with Fc-gamma-RIIIB genes that differ from the NA1-Fc-gamma-RIIIB and NA2-Fc-gamma-RIIIB by a single nucleotide. We have also shown that these single nucleotide changes affect the expression of NA1 and NA2 antigens. Analysis of the granulocytes from people with variant NA genotypes revealed that single-base substitutions in Fc-gamma-RIIIB at 141 and in Fc-gamma-RIIIB at 349 are important in the expression of NA1 and single-base substitutions in Fc-gamma-RIIIB at 227 and 277 are important in the expression of the NA2. Recent studies have focused on granulocyte antigen NB1 or CD177. The mRNA encoding CD177 has recently sequenced, and it has been found to be highly homologous to a gene over expressed on granulocytes from people with polycythemia vera, polycythemia rubra vera gene-1 (PRV-1). We searched human genomic databases to determine the genomic structure of NB1. We found only one gene homogolous to CD177 and PRV-1, suggesting that they are alleles of the same gene. However, the sequence of CD177 was incomplete. A sequence gap was present in this gene. The mRNA encoding CD177 has been cloned from a fetal liver cDNA library and is being used to screen a human genome DNA library. The CD177 gene will be sequenced and the molecular basis of antigen polymorphisms will be studied.

LBC: DTM

Title: *Ex Vivo* Culture and Characterization of Dendritic Cells for Clinical Immunotherapy Trials

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Elizabeth J. Read, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborators, Lab: Charles S. Carter (CELL, CC)
Kenneth A. Hines, BS (CELL, CC)
Janet L. Lee, B.S. (CELL, CC)

Collaborators, NIH: Jay A. Berzofsky, M.D., Ph.D. (MIVRS, NCI)
John Janik, M.D. (ID, NCI)
Samir N. Khleif, M.D. (MB, NCI)
Crystal Mackall, M.D. (NCI)
John Charles Morris, M.D., (MB, NCI)

Total Staff Years: 1

Human Research: Human subject research: cells or tissues

Keywords: Dendritic Cells, Immunotherapy

Summary: The goal of this project is to develop and evaluate methods for manufacturing dendritic cells (DCs) for clinical immunotherapy trials. In FY1999, we developed and optimized a full-scale GMP method for 5-day flask culture of autologous DCs in RPMI, autologous plasma or allogeneic serum, IL4 and GMCSF, starting with peripheral blood monocytes collected by apheresis and purified by elutriation. The immature DCs generated are then available for further manipulations (e.g., peptide pulsing) prior to clinical administration. This manufacturing method was incorporated into several clinical trials, and a manuscript describing this method was published in early 2001. Because of our interest in developing closed systems and eliminating reagents that are difficult to standardize, we evaluated a 7-day culture system in a protein-defined, serum-free medium (XVIVO15) starting with monocytes from elutriation vs. negative immunomagnetic selection using the Isolex 300I, in bags vs. flasks. We demonstrated that the two different isolation methods for monocytes produce equivalent immature DC populations, and that bags were equivalent to flasks. Furthermore, historical comparison showed that serum-free medium was equivalent and perhaps even superior to serum-containing medium for generation of immature DCs. A manuscript describing this work has been accepted for publication. Studies over the past year focused on evaluating culture conditions for generating mature DCs using CD40 ligand after culture in IL4 and GMCSF. A process was successfully developed and incorporated into several cancer immunotherapy trials in early 2001. Studies over the next year will focus on characterizing these DCs by flow cytometric phenotyping and on evaluating additional aspects of the manufacturing process, including stability of the source material (apheresis MNCs) and of the DC product after peptide pulsing.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002099-03

LBC: DTM

Title: Kinetics of Granulocyte Colony-stimulating Factor-induced Granulocyte Mobilization

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: David Frank Stroncek, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborator, Lab: Susan Leitman, M.D. (DTM, CC)

Total Staff Years: .3

Human Research: Human subject research

Keywords: Granulocyte Colony-stimulating Factor-induced Granulocyte Mobilization

Summary: The administration of granulocyte colony-stimulating factor (G-CSF) to increase the white blood cell count in granulocyte donors prior to donation is becoming an increasingly common practice. G-CSF is given subcutaneously to the donor on the day before donation, generally 12 to 24 hours before the start of apheresis. It would be advantageous to be able to give G-CSF and collect granulocytes on the same day. However, the single most important factor in optimizing granulocyte collection is the donor's precollection granulocyte count. The purpose of this study is to assess granulocyte counts in healthy subjects during an 8-hour period after a single 5 mcg/kg intravenous or subcutaneous dose of G-CSF with or without dexamethasone. Sixteen subjects were studied. Each donor was studied four separate times. The four mobilization protocols were G-CSF 5 mcg/kg given intravenously, G-CSF 5 given mcg/kg subcutaneously, G-CSF 5 mcg/kg given intravenously plus dexamethasone 8 mg given orally, and G-CSF 5 mcg/kg given subcutaneously plus dexamethasone 8 mg given orally. The order of the route of administration was assigned randomly. White blood cell and granulocyte counts were measured before G-CSF administration and at ½, 1, 2, 4, 6, 8, and 24 hours after administration. The granulocyte counts measured within the first 8 hours after G-CSF were compared with counts measured 24 hours after G-CSF. We found that granulocyte counts 8 hours after the administration of the agents were similar to the counts after 24 hours, granulocyte mobilization with intravenous and subcutaneous G-CSF was similar, and granulocyte mobilization with G-CSF plus dexamethasone was better than mobilization with G-CSF alone.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002101-03

LBC: DTM

Title: Therapy of von Willebrand's Disease with Single-donor Cryoprecipitate Collected by Apheresis

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

Supervisor of Record: Harvey Klein, M.D.

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Von Willebrand's Disease, Apheresis, Cryoprecipitate

Summary: Von Willebrand's disease (vWD) is the most common inherited bleeding disorder. We have studied the efficacy and feasibility of treating a child with type III severe vWD solely with cryoprecipitate prepared by repeated DDAVP-stimulated plasma exchange donation from a single, dedicated paternal donor. An infant presented with massive hemorrhage at circumcision. The child was found to have FVIII:C 4 percent, FVIII:Ag 20 percent, vWF:RCo 21 percent, and vWF:Ag 3 percent, indicative of severe vWD. His father carried an allele with a defect at the level of vWF mRNA expression but had a negative bleeding history with normal coagulation values. Cryoprecipitate was prepared from serial DDAVP-stimulated plasma exchange donation by the patient's father, using peripheral venous access, ACD-A anticoagulant, and autologous cryoprecipitate depleted plasma as replacement fluid. During the first 14 years of the patient's life, the father underwent 53 plasma exchange donations, yielding a total of 139,307 units of FVIII. In the past year, plasma exchange donation was standardized to involve processing of exactly 4,500 mL of plasma, which yielded a mean of 14 bags of cryoprecipitate, each having a FVIII content of approximately 330 units. Repeated plasma exchange donation was well tolerated, with adverse effects including mild headache and flushing due to the DDAVP and citrate toxicity. Cryoprecipitate was stored for up to 102 months at -70ºC. Ninety percent of the cryoprecipitate was transfused after 1 year of storage, with a mean collection-to-transfusion interval of 2.4 years. Cryoprecipitate tested after 13 to 77 months of storage showed 48 to 124 percent of the original FVIII activity; decreased activity was noted with increasing length of storage. Manufacture of plasma exchange donation-derived FVIII resulted in an estimated 50 percent cost reduction compared with similar doses of commercial factor concentrates. All bleeding episodes that occurred in the patient since birth were managed with cryoprecipitate derived by this method. At age 14, the child has received only one donor exposure throughout his life, that of the paternal donor of his cryoprecipitate. Cryoprecipitate prepared by repeated plasma exchange donation of a vWD carrier provided excellent hemostatic function, even after prolonged storage intervals of greater than 1 year. Plasma exchange donation of a committed donor may be the safest option for long-term management of vWD and provides a cost-effective alternative to commercial factor concentrates.

LBC: DTM

Title: Malarial Anemia

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: David Frank Stroncek, M.D.

Supervisor of Record: Harvey Klein, M.D.

Total Staff Years: .1

Human Research: Human subject research

Keywords: Malarial Anemia

Summary: Malaria remains a significant health problem in tropical countries. Malaria due to *Plasmodium (P) falicparum* is the leading cause of death in African children less than 5 years of age. In holoendemic areas, such as sub-Saharan Africa, severe malarial anemia is the leading cause of death in children less than 3 years of age. In severely anemic children, hemolysis is too high a risk to be accounted for by the destruction of *P. falciparum*-infected red blood cells (RBCs) because only a small fraction of all red blood cells are infected. The anemia appears to be due to hemolysis of uninfected RBCs and to be immune mediated. Severely anemic children with *P. falciparum* infections often have a positive direct antiglobulin test (DAT) due to RBCs coated with IgG and complement. *P. falciparum* infections also induce changes in RBC membranes. The expression of complement regulatory proteins CR1 (CD35) and decay accelerating factor (CD55) is decreased on RBCs from children with severe *P. falciparum* anemia, but the expression of the RBC membrane inhibitor of reactive lysis (CD59) is increased. These results suggest that both autoantibodies and RBC membrane changes may contribute to the severe malaria anemia of childhood. The purpose of these studies is to investigate the role of the immune response and RBC membrane changes in malarial anemia. In collaborative studies with Dr. Alan Magill, NIAID, we found that the culture of RBCs with *P. falicparum* had no effect on the expression of antigens by uninfected RBCs. In collaboration with Dr. Trevor Jones, U.S. Navy, malarial anemia is being studied in an aotus monkey model. Monkeys previously immunized by infection or vaccination to *P. falciparum* protein EBA-175 and challenged with low levels of parasites became severely anemic despite low levels of parasitemia. The DATs in the anemic animals were negative, suggesting a role for the spleen and cellular immune response in the anemia. Future investigations will use the *P. falicparum* vaccinated aotus monkeys as a model to further define the role of the immune system in severe malarial anemia.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002103-02

LBC: DTM

Title: Adoptive Immune Therapy for Cytomegalovirus Infection

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: David Frank Stroncek, M.D.

Supervisor of Record: Harvey Klein, M.D.

Total Staff Years: 1

Human Research: Human subject research

Keywords: Cytomegalovirus Infection, Immune Therapy

Summary: Cytomegalovirus (CMV) infections remain a serious problem in hematopoietic stem cell transplant patients. Following transplantation, CMV infections can cause pneumonititis, hepatitis, enteritis, and marrow failure. CMV seropositive transplant recipients can be treated with antiviral agents such as ganciclovir at the onset of infection or at the time of stem cell engraphment, but ganciclovir therapy is associated with renal toxicity and suppression of neutrophil counts. Preliminary studies have found that adoptive immune therapy using CMV-reactive cytoxic T lymphocytes (CTL) may be an effective and less toxic alternative to prevent CMV infection in seropositive recipients of marrow transplants. The purpose of this study is to develop new treatment strategies for producing CMV-reactive CTLs that can be used for adoptive immune therapy and to better understand the cellular immune response to CMV. Current studies are focused on identifying the immunodominant peptides that can be used to stimulate CMV-reactive CTLs. CMV contains over 200 proteins, but one protein, pp65, is the most immunogenic. Within pp65, CTLs from HLA-A*0201 people recognize only a single peptide, a nanomer pp65 495-503. We have found that for HLA-A*2401 people, the immunodominant peptide is pp65 328-337. We are working to identify the immunodominant pp65 peptides for people with types HLA-A*0101 and HLA-A*0301. HLA-A*0201 and HLA-A*2401 tetramers are being produced to use with pp65 495-503 and pp65 328-337 to follow the immune response of hematopoietic stem cell transplant patients with CMV infections.

LBC: DTM

Title: Molecular Testing Standards and New Amplification Approaches

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James Waikuo Shih, Ph.D.

Collaborator, Lab: Richard Y. Wang (DTM, CC)

Total Staff Years: .2

Human Research: Neither human cells nor tissues

Keywords: Molecular Testing Standards

Summary: This program has two components. The first is to produce an ideal internal standard that resembles the target encapsulated viral particles being tested. In order to control all processing steps in molecular detection, this standard should have the same composition of target sequence and use the same primer set for amplification. This internal standard would be most valuable in ensuring the validity of negative specimens during large-scale screening assays. The second component is to develop a new molecular amplification platform by combining two independent technologies using Mut-Y mismatch enzyme and TCR target cycling-based amplifications. These two technologies were brought together by a three-way CRADA. The end point for this collaboration is to develop a prototype test and to demonstrate its utility with clinical specimens. We have constructed a particulate HCV internal standard (IS) based on murine amphotropic retrovirus. To achieve this, we went through a stepwise process, including mutating HCV genome by inserting a 36 nucleotide base at nt 272 position in the 5-UTR and then creating a retroviral vector clone pXT-HCV-NCC-D8 containing 948 bases of the HCV sequence. This vector was used to transfect a retrovirus packaging cell line, PA317. From the transfected cells, G418-resistant recombinant retrovirus producer clones were established and further characterized. Using sequence-specific primers, we were able to show that HCV sequence-containing particles were produced. Both wild-type clones with HCV sequence and mutant clones with additional inserts were prepared and isolated. We were able to determine the insertion sequence length as expected by gene analyzer. One preparation of virus supernatant from a high virus producer, D8-54, was evaluated extensively. The relative copy number per milliliter was determined by different methods, including RT/PCR titer, electron microscope particle counting, infectious colony-forming counts, and end-point infectious titer. We found consistent results with different methods of determination. This demonstrated that this approach could provide ideal particulate IS for HCV. We were able to apply this IS to a small-scale study with clinical specimens. We were also able to develop a convenient EIA detection system based on insertion specificity. NIH filed a U.S. patent based on this work. For the second part of this project in finding new amplification approaches, in collaboration with Dr. Hsu of the University of Maryland, we found unique substrate specificity of Mut-Y enzyme that can recognize both DNA and RNA mismatches. The release mechanism for enzyme-substrate complex was determined. The cofactors, which enhance the turnover of substrate-product, were found. Potential target sequences on different strains of HIV were selected and specific probes were designed and synthesized. Ten- to thousandfold amplification was demonstrated by estimating the probe products. Specificity was shown by a narrow range of strain-specific recognition. A U.S. patent was filed jointly by the University of Maryland and NIH based on these observations and is pending. To commercialize this patent, an industry partner capable of developing this technology was sought. Medical Analysis System of Camerillo, California, presented the target cycle reaction (TCR) technology as an ideal partner. Combination between Mut-Y enzyme and TCR was shown to be compatible. A high level of amplification was demonstrated. A U.S. patent based on the conditions set forth by the CRADA is being prepared for this combined technology.

LBC: DTM

Title: Characterization of Human Pathogenic Mycoplasma from HIV-infected Patients

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: James Waikuo Shih, Ph.D.

Collaborator, Lab: Richard Y. Wang (DTM, CC)

Collaborator, Extramural: Shyhching Lo, Ph.D., M.D., Department of Geographic Pathology, AFIP

Total Staff Years: .2

Human Research: Neither human cells nor tissues

Keywords: HIV, Human Pathogenic Mycoplasma

Summary: This project is part of a long-term collaborative effort between this laboratory and Dr. Shyh-Ching Lo's lab at the Armed Forces Institute of Pathology (AFIP) to investigate the cofactors contributing to the pathogenesis of AIDS. It has been a fruitful scientific and intellectual collaboration. The laboratory continues to support the work on diagnosis and characterization of mycoplasma originating from AIDS patients, sexually transmitted disease (STD) patients, and others. We applied serological tests that were developed in this laboratory to patients in several clinical settings, including patients with HIV infection, nongonococcal urethritis (NGU), STDs, and intravenous drug use. We found a high prevalence of antibodies to *M. penetrans* in patients with Kaposi's sarcoma and antibodies to *M. genitalium* in patients with NGU. Using the paired donor-recipient specimens, we also found that *M. fermentans* and *M. genitalium* were transmissible through blood transfusion. We were able to show that the association of the presence of antibodies to *M. genitalium* with the sexual transmission of HIV was highly significant, while agents for other STDs were not. In recent years, we were asked to support Dr. Lo's lab by providing serological tests on specimens from patients who suffered from the Gulf War syndrome or Gulf War infection (GWI). Over 6,000 paired specimens, including controls, were examined. We were not able to find any difference between soldiers who served in the Gulf War and their controls in antibody seroconversion to *M. fermentans*. To clarify a report that more than 50 percent of veterans with GWI had *M. fermentans* (strain incognitus) in their blood as measured by a molecular diagnostic technique called nuclear gene tracking, we conducted a large-scale, case-control study to compare the prevalence of antibodies to *M. fermentans* lipid-associated membrane proteins (LAMPs) between the Gulf War veterans with unexplained illness and a randomly selected, matched group of veterans who did not enroll in the registry for health evaluation. In addition, we analyzed, using banked serum samples obtained on each individual before and after the deployment, the rates of seroconversion for this mycoplasma in these two groups of veterans. Our results showed that 4.8 percent of the cases and 5.2 percent of the controls tested positive for *M. fermentans*-specific antibodies before operation deployment. Most important, there was no difference in rates of seroconversion between cases and controls (1.1 vs. 1.2 percent) to *M. fermentans* during Operation Desert Storm (ODS). Thus, there is no serological evidence that suggests infection by *M. fermentans* is associated with development of GWI. We also studied blood, urine, oral swabs, and rectal swabs for evidence of

mycoplasmal infection by culture from a group of 149 Gulf War veterans who complained of various illnesses and were enrolled in the second phase of the health evaluation by the Army Comprehensive Clinical Examination Program (CCEP). None of the urine samples, oral swabs, or rectal swabs grew *M. fermentans*. No mycoplasma organism was isolated from any of the 149 blood samples. PCR study was conducted using RW oligonucleotide primer set (RW004 and RW005), based on the unique sequence of *the M. fermentans* insertion-sequence-like element. The amplified products were confirmed by southern blot, using RW006 as the hybridization probe. Each sample was tested *in triplicate at least three times.* Three out of 65 (4 percent) blood samples were considered positive. Two of these three patients tested positive for *M. fermentans* antibodies in the serological study. In conclusion, our culture study of ODS veterans with GWI revealed isolation of only mycoplasma organisms commonly found in similar samples from healthy individuals. No unusual mycoplasma was identified. Contrary to reported studies from some other laboratories, our PCR and serological studies showed only a low percentage of the veterans having evidence of *M. fermentans* infection. One of the future interests for mycoplasma study is its contribution to the development of neoplasms after long-term, low-level chronic infection. We have shown that some species of mycoplasma were able to transform cells *in vitro* after long-term co-cultivation, and several indicative oncogenes were activated.

LBC: DTM

Title: Prospective Studies of Phlebotomy Therapy in Hereditary Hemochromatosis

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborators, NIH: Charles Bolan (DTM, CC)
Janet N. Browning (CC)
Yu Ying C. Yau (CC)

Total Staff Years: .4

Human Research: Human subject research: cells or tissues

Keywords: Phlebotomy Therapy, Hereditary Hemochromatosis

Summary: The recent availability of a genetic test (homozygosity for the C282Y mutation in the HFE gene) for the diagnosis of hereditary hemochromatosis (HH) has focused renewed attention on this relatively common disorder. However, phlebotomy therapy in HH remains hampered by lack of simple, physiologic laboratory monitoring guides. In addition, phlebotomy therapy has been perceived as wasteful because the blood obtained is not used for allogeneic transfusion, although many HH subjects meet standards for allogeneic blood donation. Recent regulatory changes now allow increased flexibility in establishing policies for transfusion of blood obtained from HH subjects. We developed a protocol for use of the red cell mean corpuscular volume (MCV), a precisely measured indicator of erythopoietic iron availability, as a simple, physiologically based target to guide the pace of induction and maintenance phlebotomy for HH. We also developed a program to use blood therapeutically withdrawn from HH subjects for allogeneic transfusion. To enable the operational aspects of using HH donor blood for transfusion, a customized multi-user database program was developed as a Microsoft Access application to maintain and analyze lab data, generate a schedule of phlebotomy intervals and appointment dates, and notify staff when preset therapeutic end points were reached. We enrolled 60 patients with HH in the first year of this protocol. Induction phlebotomy to achieve iron depletion was performed every 1 to 4 weeks, depending on subject weight and initial ferritin levels, and continued until the MCV decreased by 3 to 5 percent below pretreatment baseline. A finger-stick hemoglobin (HGB) greater than 12.5 g/dL was used as the threshold for performing phlebotomy. Maintenance phlebotomy was targeted to maintain the red cell MCV at 3 percent below baseline, with weekly to monthly measurements of MCV, ferritin, and transferrin saturation (TS) to determine the rate of reaccumulation of iron. Median pretreatment values in the first 27 previously untreated patients included ferritin 1039 (range 65 to 5248) ng/mL, TS 80 (32 to 95) percent, and MCV 96.3 (90 to 105) cubic microns. Median ferritin was 16 (5 to 47) ng/mL, TS was 11 (3 to 22) percent, and HGB was 12.0 (10.9 to 13.3) g/dL at the point of transition from induction to maintenance therapy, as defined by the MCV guide. A mean of 19 induction bleeds were performed until iron depletion was achieved. Nadir HGB levels of 11.7 (10.4 to 12.6) g/dL occurred 1.5 (0 to 4) weeks after the transition to maintenance therapy. The mean iron removal necessary to maintain a stable ferritin, MCV, and TS during maintenance therapy in 22 C282Y homozygotes was 50 ug/kg/day, was highly correlated with body weight,

and was significantly higher in the C282Y homozygotes than in five C282Y/H63D compound heterozygotes, 33 ug/kg/day. Women and older subjects tolerated initial induction phlebotomy better at 2- rather than 1-week intervals. These data correspond to a stable maintenance interval of every 7 to 9 weeks for an 85 kg C282Y/C282Y homozygote, and every 10 to 12 weeks for a compound heterozygote of similar size using 500 mL whole blood phlebotomy and a targeted maintenance HGB of 14 g/dL. Thirty-three of 55 (60 percent) of HH subjects had arthritis on entry, but there was no definite improvement in joint complaints with progressive iron depletion. Occurrence of arthritis was highly correlated with higher iron burden at presentation and with C282Y homozygosity. Forty-six (77 percent) of the HH subjects met donor eligibility criteria. During this period, 256 red cell units derived from HH subjects entered allogeneic inventory, constituting 6 percent of all allogeneic red cells collected at our center. Positive viral markers were found in 4 HH subjects, all of whom admitted deferrable risk prior to testing. Subjects expressed great satisfaction in knowing their blood was made available to others rather than discarded. Use of the red cell MCV provides an inexpensive, simple, and individualized parameter that is widely available and suitable for use to achieve optimal phlebotomy therapy. Our data indicate that serial MCV changes reliably indicate iron depletion and can be used to avoid symptomatic anemia at the transition to maintenance phlebotomy. HH subjects can safely augment the allogeneic blood supply, leading to improvements in allogeneic inventory, in HH patient care, and in benefit to the community. Our data argue strongly for a comprehensive movement of HH phlebotomy care into the blood center. Phlebotomy can be simply and safely managed in this setting with the use of HGB- and MCV-based guidelines.

LBC: DTM

Title: Massive Immune Hemolysis in Blood Stem Cell Transplants with Minor ABO Incompatibility

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Susan Leitman, M.D.

Supervisor of Record: Harvey Klein, M.D.

Collaborators, NIH: Charles Bolan (DTM, CC)
Richard W. Childs, M.D. (HB, NHLBI)

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Peripheral Blood Stem Cell Transplants, Immune Hemolysis, ABO Incompatibility

Summary: Massive immune hemolysis due to minor ABO incompatibility is an underappreciated, potentially fatal complication of allogeneic hematopoietic transplantation. The increased lymphoid content and rapid engraftment seen with peripheral blood stem cell (PBSC) transplants may increase the frequency and severity of this event. In addition, nonmyeloablative conditioning regimens favor rapid and vigorous donor-type immune reconstitution, relying on donor lymphocytes to mediate both an anti-tumor effect and durable myeloid engraftment. To further the graft vs. tumor effect, antiproliferative agents such as methotrexate are frequently omitted from posttransplant anti-GVHD regimens. We observed abrupt, catastrophic hemolysis in the first NIH patient to receive a nonmyeloablative PBSC transplant involving minor ABO incompatibility. We established a protocol for close clinical and laboratory monitoring of the next nine consecutive minor ABO-incompatible, nonmyeloablative PBSC transplants performed on NHLBI and NCI services. Cyclosporine alone was employed to prevent GVHD in all nine cases. Two additional cases of massive immune hemolysis were detected. Hemolysis began 7 to 11 days following stem cell infusion. Both cases responded rapidly to vigorous hydration and prompt donor-compatible red cell transfusions, without adverse clinical consequences. All patients with hemolysis demonstrated a positive direct antiglobulin test (DAT), with eluate reactivity against the relevant recipient blood group (anti-A in two cases, anti-B in one). However, neither the intensity of the DAT nor the donor isohemagglutinin titer distinguished cases with from those without hemolysis. These results demonstrate that isohemagglutinins produced by donor passenger B lymphocytes in minor ABO-incompatible PBSC transplants utilizing cyclosporine alone for GVHD prophylaxis can mediate massive immune hemolysis in a considerable proportion of subjects at risk. In view of this high risk, anti-GVHD regimens in NHLBI protocols were changed to include mycophenolate mofetil (MMF), an antiproliferative agent. None of the next ten consecutive minor ABO-incompatible nonmyeloablative stem cell transplants were accompanied by significant immune hemolysis, although serologic abnormalities were seen. GVHD regimens continue to be modified to maximize graft antitumor immune effects while minimizing other immune complications of transplant, and MMF doses are being reduced in an effort to increase complete remission rates post-transplant. We continue to monitor daily blood counts and red cell serologic studies (DAT, IAT) during the period at risk (day 6 to day 11 post-transplant) and to promptly administer donor-compatible red cell transfusions in these cases. Improved awareness can avert serious complications due to minor ABO incompatibility following stem cell transplant and should be practiced in all such cases.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002110-01

LBC: DTM

Title: Transfusion-related Infections Prospectively Studied (TRIPS)

Dates: from 10/01/2000 to 09/30/2001

Principal Investigator: Harvey J. Alter, M.D.

Collaborators, Lab: Mary Helen Boone (IDS, CC)
Pamela L. Hernandez (IDS, CC)
Harvey Klein, M.D. (IDS, CC)
Cathy A. Schechterly (IDS, CC)
James Waikuo Shih, Ph.D. (IDS, CC)
Bernice L. Williams (IDS, CC)

Collaborator, Extramural: Michael Busch, M.D., Ph.D., Department Transfusion Medicine
Blood Centers of the Pacific

Total Staff Years: 5

Human Research: Human subject research: cells or tissues

Keywords: Hepatitis, Blood Transfusion, Adverse Events, Microchimerism, Viruses

Summary: Improved viral screening assays and more intensive questioning of donors for high-risk behaviors have resulted in dramatic declines in the rates of transfusion-transmitted hepatitis and AIDS. Nonetheless, there is a need for continued vigilance to ensure the safety of blood supply. This study will enroll blood donors and prospectively followed blood recipients in order to (1) establish ongoing surveillance of the incidence of breakthrough infections from transfusion-transmitted agents for which there are existing donor-screening assays (e.g., HBV, HCV, HIV, human T-cell lymphotropic virus [HTLV]); (2) monitor the transfusion risk of established infectious agents that are not routinely screened in blood donors, including CMV, EBV, parvovirus B-19, HHV-8 (Kaposi's sarcoma virus), and the recently described SEN and TT viruses (possible hepatitis agents); and (3) establish a repository of linked donor and recipient samples so that any newly emerging infectious agent can be rapidly evaluated for its threat to the blood supply. The risk of these blood-transmitted infections will be assessed by molecular and serologic assays in adult patients at NIH and in children at Children's National Medical Center. Blood samples from recipients transfused on one occasion will be obtained pre-transfusion and 4, 8, 12, and 24 weeks post-transfusion. Recurrently transfused patients will have additional samples at 16 and 20 weeks after the index transfusion and 24 weeks after the last eligible transfusion. After initial infectious disease testing, recipient samples and linked donor samples will be stored in a repository maintained by the National Heart, Lung, and Blood Institute. The availability of the repository will allow for the assessment of transfusion risk for newly emerging pathogens and also for known agents for which no practical assay is currently available. For example, this would allow future testing for prions in new variant Creutzfeld-Jacob disease (human variant of mad cow disease) or testing for the trypanosome that causes Chagas disease. Informed consent will be obtained to store and later test samples in the repository. Testing will be limited to infectious agents that potentially threaten the blood supply. No genetic testing will be performed.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002111-01

LBC: DTM

Title: RBC Leukocyte Reduction Filter Failures in Blood Donors with Sickle Trait

Dates: from 10/01/2000 to 09/30/2001

Lead Investigator: David Frank Stroncek, M.D.

Supervisor of Record: Harvey Klein, M.D.

Total Staff Years: .2

Human Research: Human subject research

Keywords: Sickle Cell Trait, RBCs, Leukocyte Reduction Filters

Summary: Fifty to 75 percent of RBC components from donors with sickle cell trait obstruct leukocyte reduction filters. People with sickle cell trait are healthy, but very low oxygen levels, low pH, and high hemoglobin concentrations can induce intracellular hemoglobin S polymerization. We have found that hemoglobin S polymerization, due to low oxygen tension in venous blood and low pH and high osmolarity of the citrate anticoagulant, is responsible for the failure of RBC components from donors with sickle cell trait to filter. The purpose of ongoing studies is to determine if the failure of sickle cell trait donor blood to filter can be avoided by improving the oxygenation of blood prior to filtration, reducing the exposure of RBCs to citrate, or a combination of both. The oxygenation of blood will be improved by storing blood in gas-permeable bags prior to filtration and using gas-permeable flow paths to oxygenate blood as it is collected. The effects of citrate anticoagulant on filterability will be assessed by comparing blood collected by phlebotomy with blood collected by apheresis, which reduces citrate exposure by approximately 50 percent. RBC components collected from donors with sickle cell trait by both phlebotomy and apheresis will be collected with gas-permeable flow paths and in gas-permeable bags, and their filterability will be tested. The goal of these studies is to develop a practical method to allow the successful leukocyte reduction by filtration of all RBC components collected from donors with sickle cell trait. The method should be easy to use in conjunction with existing blood collection technologies.