The Warren Grant Magnuson
Clinical Center

Annual Report of Clinical Research Activities

October 1, 2001 to September 30, 2002

NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT OF CLINICAL RESEARCH ACTIVITIES

October 1, 2001 to September 30, 2002

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

For Administrative Use

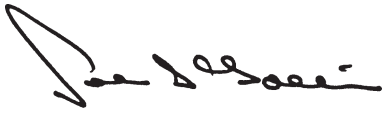
DIRECTOR'S MESSAGE

The Clinical Center's mission includes the provision of "…patient care, services, training and the environment in which NIH clinician-scientists creatively and ethically translate emerging knowledge to improve the detection, treatment and prevention of human diseases for the health of a diverse nation." In support of this mission throughout FY 2002, Clinical Center departments responded to institute requests for expanded research initiatives including the development of new programs in transplantation, tumor vaccine research, and enhanced imaging sciences services.

This past year NIH Clinical Center researchers were recognized for their own research accomplishments. Harvey Alter, M.D., Chief of the Infectious Diseases Section and Associate Director of Research within the Department of Transfusion Medicine, became the first Clinical Center scientist to be elected to both the National Academy of Sciences in April 2002 and the Institute of Medicine in October 2002. His work in transfusion-related hepatitis led to the discovery of the hepatitis C virus. Last year Dr. Alter won the Lasker Award for his work in cleansing the Nation's blood supply of hepatitis infection.

Significant advances with technology transfer were made this year by researchers within the Clinical Center. For example, the Sequential Lipoprotein Lipid Test, developed in the Department of Laboratory Medicine, can be used to measure HDL-cholesterol, total cholesterol and triglycerides in a single tube without any specimen pre-processing, such as centrifugation. Because these three lipid fractions are used for calculating LDL-cholesterol, the new test represents a significant simplification of the current laboratory procedure for assessing coronary artery disease risk and also significantly reduces the overall cost for the testing. Genzyme Diagnostics, one of the largest producers of reagents for lipoprotein lipid tests, received an exclusive license and plans to commercialize the Sequential Lipoprotein Lipid Test for distribution to the major diagnostic companies that manufacture automated clinical chemistry analyzers.

Through a sustained commitment to excellence in clinical research, Clinical Center staff continues to facilitate innovative research activities. This report summarizes clinical research projects overseen by Clinical Center investigators in FY 2002.



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 CL008047-01

LBC: ANES

Title: The Role of Nitric Oxide Synthase 1 in a Rodent Model of Sepsis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Zenaide Quezado, MD (CC)

Supervisor of Record: Henry Masur, MD (CCM, CC)

Collaborator, Lab: Peter Q. Eichacker, MD (CC)

Total Staff Years: .3

Human Research: Neither human cells nor tissues

Keywords: Sepsis, peritonitis, infection, nitric oxide, NOS1, nNOS, mice, knock out

Summary: Several studies have shown that nitric oxide (NO), a free radical gas produced endogenously, has profound effects on the function of leukocytes and in the overall host immune response to infection, sepsis, and septic shock. In cells, NO is synthesized by three different isoforms of an enzyme named NO synthase (NOS). Two of these isoforms, NOS1 and NOS3, are constitutively expressed, while the third, NOS2, is an inducible (by toxins and cytokines) form of the enzyme. Differing lines of evidence suggest that NOS1 may have a very important role in the pathophysiology of sepsis. The enzyme is constitutively expressed not only in neuronal cells in the brain and spinal cord, but also in the microvasculature and epithelium of the gastrointestinal tract and kidney, bronchial epithelium, myocytes of skeletal muscle, mast cells in skin, and neutrophils. Researchers have shown that, under baseline conditions and during sterile peritonitis, mice congenitally lacking NOS1 (NOS1 knock out, NOS1-KO) have increased leukocyte rolling and adhesion to the endothelium of postcapillary venules and increased leukocyte migration into the peritoneal cavity. The purpose of this study is to investigate the effects of NOS1 on extravascular neutrophil recruitment, bacterial clearance, and inflammatory tissue injury during polymicrobial peritonitis, sepsis, and septic shock. We hypothesize that absence of NOS1, either in genetically engineered mice that congenitally lack the NOS1 gene or in wild type mice (phenotype considered normal) treated with a pharmacologic inhibitor of NOS1, will increase extravascular neutrophil recruitment and improve bacterial clearance and outcome during polymicrobial peritonitis and sepsis. Following cecal ligation and puncture in wild type or NOS1-KO mice randomized to receive a NOS1 inhibitor or placebo, survival, extravascular neutrophil recruitment, microbial clearance, and inflammatory tissue injury will be measured.

CLINICAL BIOETHICS DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL010501-04

LBC: CB

Title: Survey of Organ Procurement Organizations

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators, Lab: Neal Dickert, BA (CBD, CC)
David S. Wendler, PhD (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 NHLBI 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 the Office of Management and Budget's approval. Approval was granted. Sixty-one individuals were surveyed, and a manuscript has been published.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010502-04

LBC: CB

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborator, Lab: David S. Wendler, PhD (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. The study was approved by the NIMH IRB. We surveyed 504 individuals. Several papers are either in press or have been published.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010505-04

LBC: CB

Title: Patient Perspectives on Health Insurance

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Marion Danis (CBD, CC)

Supervisor of Record: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators, Extramural:
Richard Duke, PhD (Multilogue Corporation, Richard Duke & Associates)
Susan Dorr Goold, MD, MSHA, MA (Regents of University of 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 pilot tested. The study design has been completed. Study subjects were recruited and 50 group exercises have been conducted. Data collection was completed on June 23, 2000. Data analysis is also complete. A manuscript has been published. The tool has been developed into a computer-based exercise which has been copyrighted by the University of Michigan with joint ownership by NIH.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010506-04

LBC: CB

Title: Defining a Health Insurance Package for the Uninsured

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Marion Danis (CBD, CC)

Supervisor of Record: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators Extramural:
Andrea Biddle, PhD, MPH, PhD, MPH
(Health Policy and Administration, UNC – Chapel Hill)
Susan Dorr Goold, MD, MSHA, MA
(Regents of University of 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. The study instrument was designed and pilot tested, study subjects were recruited, and 20 group exercises were conducted. Data collection and data analysis are complete. Two abstracts have been presented at national meetings. A manuscript was published in February 2002.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010507-04

LBC: CB

Title: Physician Resolution of Ethical Problems

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Marion Danis (CBD, CC)

Supervisor of Record: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators, Extramural: Brian Mi Clarridge (Center for Survey Research, University of Massachusetts)
Gordon DuVal, SJD, SJD (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 is complete. 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 CL010508-04

LBC: CB

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators, Extramural: Arlene Ash, PhD (Health Care Research Unit, Boston University)
Gail Gazelle, MD (Harvard Pilgrim Health Care)
Wei Yu, PhD (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 resources 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 65 and those under 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 complete, and 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 that are provided, differences in site of death, and causes of death using 2000 data.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010509-04

LBC: CB

Title: Protecting Communities in Biomedical Research

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborator, Extramural: Charles Weijer, MD, PhD (Office of Bioethics Education and Research, Dalhousie University)

Total Staff Years: 2

Human Research: Neither human cells nor tissues

Keywords: Biomedical research

Summary: This two-phase project is examining protections that should be afforded to communities in research. The first phase identified and analyzed existing protections for communities. 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 they do not necessarily apply to other types of communities. The second phase focuses on determining what protections are appropriate for different types of communities. It 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 homogeneous. 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. (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. Two articles were published: Weijer C, Emanuel E. Protecting Communities in Biomedical Research. *Science* 2000; 289:1142-1144. Weijer C, Goldsand G, Emanuel EJ. Protecting Communities in Research: Current Guidelines and Limits of Extrapolation. *Nature Genetics* 1999; 3:275-280.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010510-04

LBC: CB

Title: BEST: Best Ethical Strategies in Managed Care

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

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

Collaborators, Extramural: Steve Pearson, MD, MPH, MD (Center for Ethics in Health Care, Harvard Pilgrim Health Care)
James E. Sabin, MD (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 managed care plans might adopt best practices regarding these ethical issues if they were provided with a list. A consortium of 12 managed care plans was established, including for-profit, not-for-profit, academic, "Blues," and religious-based plans. Nine different 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, ten 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. Study completed. Book contract with Oxford University Press, estimated publication in spring 2003.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010511-04

LBC: CB

Title: Managing Pharmacy Benefits in Managed Care

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

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

Total Staff Years: 1

Human Research: Neither human cells nor tissues

Keywords: Managed care

Summary: Managing pharmacy benefits is a major dilemma for managed care organizations and health insurers. 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. Study is completed. Two articles have been generated from this study.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010512-03

LBC:	CB
Title:	Minnesota CHAT: Public Perspectives on Health Insurance in Minnesota
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Marion Danis (CBD, CC)
Supervisor of Record:	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborators, Extramural:	Ellen Benavides (Health Strategies Group) Andrea Biddle, PhD, MPH (Health Policy and Administration, UNC – Chapel Hill) Susan Dorr Goold, MD, MSHA, MA (Regents of University of 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 encourage the managed care industry in Minnesota to attend to rising costs and dissatisfaction with choices in health care. The study instrument has been designed, and the study design has been completed. Participant recruitment is currently ongoing. Thirty group exercises are 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 CL010513-03

LBC: CB

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators, Lab: Christine Grady, PhD (CBD, CC)
Christine A. Pace (CBD, CC)
David S. Wendler, PhD (CBD, CC)

Total Staff Years: 1.2

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 the use of Interleukin-2 in HIV disease. Our study is interviewing four groups participating in ESPRIT: (1) chairs of the Institute Review Board (IRB) or REC that reviewed ESPRIT; (2) principal investigators (PIs) 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 about important ethical issues associated with ESPRIT. There has been substantial controversy about the ethics of research involving human subjects 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. Still conducting interviews with IRB chairs, PIs, and research participants in several countries.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010514-03

LBC: CB

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators, Lab: Christine Grady, PhD (CBD, CC)
Sam Horng, BA (CBD, CC)
Jonathan Rackoff, BA (CBD, CC)
Benjamin S. Wilfond, MD (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 were collected from all NCI-designated comprehensive cancer centers and from major pharmaceutical companies that conduct Phase I oncology trials. 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 reviewed 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) characteristics of the trial, 2) the research purpose and procedures, 3) benefits, 4) risks, and 5) alternatives. We found that overall the Phase I oncology consent forms did not overpromise benefit or downplay risk. In fact, only 1 of 272 forms said the participant could expect benefit, and most described the prospect of benefit as uncertain. The vast majority described the purpose of the Phase I trial and explained risk comprehensively, including mentioning the possibility of death.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010515-01

LBC: CB

Title: Ethical Issues Associated with Nurse Practitioner and Physician Assistant Practice

Dates: from 10/01/2001 to 09/30/2002

Principal Investigators: Christine Grady, PhD (CBD, CC)
Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborator, Lab: Marion Danis (CBD, CC)

Collaborators, NIH: Connie Ulrich, RN, PhD, (CC)
Deloris Koziol, PhD (OD, CC)

Total Staff Years: 1

Human Research: Human subject research: Interviews

Keywords: Nurse practitioners, physician assistants, ethical conflict, delivery of care, ethics preparedness

Summary: This protocol is a descriptive, cross-sectional, nationally representative study to investigate nonphysician practitioners' (NPs and PAs) perceptions of ethical issues associated with primary care practice. NPs in primary care have expressed ethical conflicts in clinical practice centering on issues of justice, rights, responsibility, nonmaleficence, and beneficence. Other ethical conflicts identified for NPs include arrangements with managed care organizations, professional accountability, pressure to see an increasing volume of patients, and bonus and billing practices to lower costs. with the projected trends for NPs and PAs substantially increasing, knowledge of factors that influence ethical practice will be relevant in shaping the future role of these practitioners in providing quality cost-effective health care. Additionally, as part of the methodological purpose of the study, subjects will be randomly assigned to one of three groups to evaluate the effectiveness of monetary incentives to increase response rates to a mailed self-administered questionnaire. Data will be analyzed using descriptive statistics and measures of central tendency (frequencies, mean, standard deviation) as well as bivariate correlations. Multivariate regression will be used to determine factors that predict ethical conflict in practice and perceived delivery of quality care. To investigate differences in response rates of practitioners related to various monetary incentives, a chi-square will be computed.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010516-01

LBC:	CB
Title:	Survey of Institutional Review Board Chairs Regarding Interpretation of U.S. Federal Pediatric Regulations
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborator, Lab:	David S. Wendler, PhD (CBD, CC)
Collaborator, NIH:	Benjamin S. Wilfond, MD (OCD, NHGRI)
Collaborators, Extramural:	Seema Shah, BA (Stanford Law School) Amy Whittle (Cornell University School of Medicine)
Total Staff Years:	1.1
Human Research:	Human subject research: Interviews
Keywords:	IRB, assent

Summary: In order to assess the protection of children who are enrolled in clinical research, it is important to determine how Institutional Review Boards (IRBs) reviewing such research interpret and implement the Federal regulations set forth in 45CFR 46 Subpart D. This study gathered this information through interviews with IRB chairpersons. The survey specifically looked at how IRBs assess risk/benefit levels of research with children, when IRBs permit children's assent to be waived, what information IRBs require to be presented to children during the assent process, and which children are excluded from participation in riskier research. In addition, the survey will attempt to determine how the recent NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects has affected IRB review. The survey will be administered to the chairpersons of the U.S. IRBs used by pediatric departments of medical schools, children's hospitals, institutes that receive NICHD funding, and also the chairpersons of the currently functioning private IRBs. This study was approved by the NICHD IRB. Surveying is completed and we are currently writing up the results.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010517-01

LBC: CB

Title: Assessing the Assent Process in Pediatric Research

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ezekiel Emanuel, MD, PhD (CBD, CC)

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

Collaborators, Extramural: Gail Geller, ScD (School of Hygiene and Public Health, Johns Hopkins University)
Steve Joffe, MD, MPH (Boston Children's Hospital)
Rick Kodish, MD (Case Western Reserve University)
Phil Rosoff, MD (Duke University School of Medicine)

Total Staff Years: 1.1

Human Research: Human subject research: Interviews

Keywords: Pediatric, assent

Summary: One of the principal safeguards mandated by the Federal regulations governing clinical research with children is the assent requirement: children who are capable must provide an "affirmative agreement" to participate unless the research "holds out a prospect of direct benefit that is important to the health or well-being of the children and is available only in the context of the research" (46.408). Despite the importance of the assent requirement, the Federal regulations offer no guidelines on its implementation. Most important, unlike the Federal regulations for obtaining consent, there are no requirements concerning what information must be given to children prior to soliciting their assent. Similarly, the Federal regulations do not provide any guidance on how the solicitation of children's assent should be coordinated with the requirement to obtain parental permission. In the present study, we propose to survey children enrolled in clinical research and one of their parents in order to obtain information about children's role in making decisions on their participation in clinical research. This study was approved by the NICHD IRB and the Duke University IRB. We are currently conducting pretest interviews.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010518-01

LBC:	CB
Title:	A Survey of European Physicians Regarding Ethical Dilemmas in Clinical Practice
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Marion Danis (CBD, CC)
Supervisor of Record:	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborator, Lab:	Samia Hurst, MD (CBD, CC)
Collaborators, Extramural:	Ruth Brown (Medtap International) Reidun Forde, MD (Norwegian Medical Association) Renzo Pegoraro, MD (Fondazione Lanza) Arnaud Perrier, MD (Hopitaux Universitaires de Geneve) Stella Reiter-Theil, MD (Institut fuer Angewandte Ethik und Medizinethik, Universitaet Basel) Anne Slowther, MD (Ethox Institute for Health Sciences)
Total Staff Years:	1.1
Human Research:	Human subject research: Interviews
Keywords:	Questionnaires, physicians, Europe, ethics, clinical, cost allocation, health care rationing

Summary: This study is a cross-sectional, self-administered mailed survey to address the type and frequency of ethical dilemmas faced by physicians, how they approach them, the types of ethical support they would find useful in addressing these ethical dilemmas, and their attitudes and practices when resources are scarce. The questionnaire has been designed, piloted, translated, and back translated. The study sample will include 400 general practitioners and general internists from each of four European countries, including England, Switzerland, Norway, and Italy. IRB approval has been attained at the NIH and in each of the participating countries. A survey firm has been selected and data collection is in planned to begin at the end of 2002.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010519-01

LBC: CB

Title: Perceptions of Benefits and Risks – Rakai

Dates: from 10/01/2001 to 09/30/2002

Principal Investigators: Christine Grady, PhD (CBD, CC)
Ezekiel Emanuel, MD, PhD (CBD, CC)

Collaborators, Lab: Elizabeth R. Wahl (CBD, CC)
David S. Wendler, PhD (CBD, CC)

Collaborators, Extramural: M. Kiddugavu, MB, ChB, MPH (The Rakai Project, Uganda Virus Research Institute)
F. Nalugoda, BSc, MHS (The Rakai Project, Uganda Virus Research Institute)
D. Serwadda, MB, ChB, MMed, MPH (Institute of Public Health, Makerere University)
J. Wagman, MHS (Heilbrunn Department of Population and Family Health, Columbia University)
Maria Wawer, MD (Columbia University)

Total Staff Years: .1

Human Research: Human subject research: Interviews

Keywords: Developing countries, multinational, ethics, survey, Africa

Summary: This protocol has been approved by the NIAID IRB (03-CC-NO39).
This study seeks to inform deliberation and resolution of ethical issues related to biomedical research in developing countries through empirical data obtained from research participants, community members, and opinion leaders in the Rakai District in southwestern Uganda.
In particular, this study will look at how people involved in research in a developing country perceive the benefits and risks to communities involved in biomedical research. The study is part of the Rakai Project, an ongoing prospective cohort study with an intensive population-based HIV/STD epidemiological, behavioral, and intervention research program in 46 communities. A representative sample of men and women will be drawn from communities in the Rakai District to include: 1) people who have participated in Rakai Project research, including both those who have received financial compensation for their participation and those who have not, 2) people who have never been asked to participate in research, 3) people who have declined to participate in research, and 4) people who are perceived as leaders in their communities. Men and women interviewed will come from deep rural, rural, and peri-urban communities. The primary methodology of the study is a quantitative survey administered to individuals; a secondary methodology is qualitative in-depth individual interviews and focus group discussions.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010520-01

LBC:	CB
Title:	Ethics Substudy of an Anti-malarial Efficacy Study in Uganda
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigators:	Christine Grady, PhD (CBD, CC) Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborators, Lab:	Christine A. Pace (CBD, CC) David S. Wendler, PhD (CBD, CC)
Collaborator, Extramural:	A. Talisuna, MD (Ministry of Health)
Total Staff Years:	.1
Human Research:	Human subject research: Interviews
Keywords:	Informed consent, multinational, stored blood samples, ethics, survey, Africa

Summary: This protocol has been approved by the NIAID IRB. This substudy seeks to inform deliberation and resolution of ethical issues related to clinical research in developing countries through empirical data obtained from parents/guardians who enrolled their children in an anti-malarial efficacy study. The anti-malarial study is being conducted at East Africa Network for Monitoring Anti-malarial Treatment (EANMAT) sites in Uganda, Rwanda, Tanzania, and Kenya. The study will evaluate the safety and efficacy of LapDap (chlorproguanil/dapsone) plus artesunate for uncomplicated malaria in children aged 3 to 59 months. It will also compare the efficacy of LapDap to that of sulphadoxine pyrimethamine plus amodiaquine. The ethics substudy will be conducted at the four Ugandan sites. Its primary methodology is individual interviews, and a secondary methodology is focus group discussions.

CRITICAL CARE MEDICINE DEPARTMENT

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LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Kovacs, MD (CCM, CC)

Collaborators, NIH: Richard Davey, Jr., MD (CRS, NIAID)
Judith Falloon, MD (CMRS, NIAID)
Henry Clifford Lane, MD (CMRS, NIAID)
Henry Masur, MD (CCM, CC)
Michael Polis, MD (CMRS, NIAID)
Jorge L. Tavel, MD (DIR, NIAID)
Diane M. Rock Kress (CC)

Total Staff Years: .2

Human Research: Human subject research

Keywords: Interleukin-2, HIV infection

Summary: Interleukin-2 (IL-2) is a cytokine with important regulatory properties for 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/mm^3, 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/mm^3. 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^3 in combination with currently approved antiretroviral therapies. The study opened in April 1993 and was completed in February 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. In addition, in combination with labeling studies, we are investigating the mechanisms leading to the profound CD4 cell increases seen with intermittent IL-2 therapy. These studies are potentially important because they are the first ones to suggest that immunomodulating agents combined with antiretroviral agents may have a benefit in patients with HIV infection.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000037-15

LBC: CCM

Title: The Characterization of *Pneumocystis carinii* Surface Antigens

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Kovacs, MD (CCM, CC)

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

Total Staff Years: .9

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. Previously we had developed an enzyme-linked immunosorbent assay (ELISA) to examine antibody responses to these antigens, Over the past year we have refined this ELISA to allow quantitation of antibody responses, and have used it to examine sera from patients with or without HIV infection and with or without a history of *P. carinii* pneumonia (PCP), as well as sera from a variety of healthy controls. In about 15 percent of healthy patients followed serially we have been able to document changes in antibody titers, suggesting that these individuals have developed reinfection or reactivation of *P. carinii* infection. 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. Within this expression site we have identified a region of tandem repeats that varies among different *P. carinii* isolates and thus provides a new method for typing human *P. carinii*. 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 CL000073-14

LBC: CCM

Title: Investigations of New Therapies in Septic Shock

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Charles Natanson, MD (CCM, CC)

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

Total Staff Years: 1.12

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 causes 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 septic shock die from the syndrome. Thus septic shock, which affects young children and the elderly alike (even those without predisposing illness), is a common and important clinical problem with substantial mortality and one that 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 syndrome to reduce morbidity and mortality will result from curing the infection and interrupting the effects of these organism and host mediators. Using purpose-bred beagles, the canine model of septic shock has 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 adults 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. Several therapies are under investigation that might be effective in human septic shock. The canine model is ideally suited for preclinical trials of these new therapies. The model allows properly controlled trials to evaluate therapeutic mechanisms and adverse effects of therapies, which is not always possible in human studies. This model is expected to be used in 2002–2004 to test the efficacy of intra-aortic balloon pumping and to compare the effects of vasopressin, norepinephrine, and a combination as a therapy for shock. Other agents to be tested include AG556, Tyrosin kinase inhibitor adjusted to the severity of illness. Anti-inflammatory agents such as anti-TNF antibodies and interleukin 1 receptor antagonists adjusted for severity of illness will also be tested.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000133-09

LBC:	CCM
Title:	Effect of Nitric Oxide Synthase Inhibitors in *in vivo* Tumor Necrosis Factor-induced Myocardial Depression
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Charles Natanson, MD (CCM, CC)
Collaborators, Lab:	Steven Solomon, PhD (CCM, CC) Steven Banks, PhD (CCM, CC) Robert L. Danner, MD (CCM, CC) Peter Q. Eichacker, MD (CCM, CC) Melinda S. Fernandez (CCM, CC) Allen T. Hilton (CCM, CC) Stephen S. Richmond (CCM, CC)
Total Staff Years:	1.57
Human Research:	Neither human cells nor tissues
Keywords:	Nitric oxide synthase inhibitors, tumor necrosis factor-induced myocardial depression
Summary:	This project has been completed. A manuscript has been submitted.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000146-09

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Kovacs, MD (CCM, CC)

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

Collaborator, NIH: Irini Sereti (DIR, NIAID)

Total Staff Years: .7

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. Over the past year we have been able to demonstrate the kinetics of *P. carinii* infection in healthy mice. We are planning to utilize gene chip studies to try to identify immune mechanisms that are important in controlling infection in these animals. 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 CL000149-09

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James H. Shelhamer, MD (CCM, CC)

Collaborator, Lab: Rafal Pawliczak, MD, PhD (CCM, CC)

Total Staff Years: .6

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 30-36 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 down-regulate basal transcriptional activity as measured by mutation and deletion reporter gene constructs. A manuscript is in preparation.

INTRAMURAL RESEARCH PROJECT

1 Z01 CL000155-07

LBC:	CCM
Title:	Study of Salvage Regimens for Patients with HIV Infection Demonstrating Virologic Failure with Licensed Protease Inhibitors
Dates:	from 10/01/2001 to 12/31/2001
Principal Investigator:	Henry Masur, MD (CCM, CC)
Collaborators, NIH:	Judith Falloon, MD (CMRS, NIAID) Henry Clifford Lane, MD (CMRS, NIAID) Susan Vogel, RN (CMRS, NIAID)
Total Staff Years:	1
Human Research:	Human subject research: cells or tissues
Keywords:	HIV infection, virologic failure, protease inhibitors
Summary:	This project is completed.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000164-07

LBC:	CCM
Title:	A Controlled Trial of Tyrosine Kinase Inhibitors in a Canine Model of Septic Shock
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Charles Natanson, MD (CCM, CC)
Collaborators, Lab:	Steven Solomon, PhD (CCM, CC) Robert L. Danner, MD (CCM, CC) Peter Q. Eichacker, MD (CCM, CC) Michael A. Solomon, MD (CCM, CC)
Total Staff Years:	1.1
Human Research:	Neither human cells nor tissues
Keywords:	Tyrosine kinase inhibitors, canine model, septic shock
Summary: for this Annual Report.	This project has been completed, currently there is no bibliography

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000165-07

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Frederick P. Ognibene, MD (CCM, CC)

Collaborators, Lab: Mark T. Gladwin, MD (CCM, CC)

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

Collaborator, Extramural: Anthony Slonim, MD (Critical Care Medicine, Children's National Medical Center)

Total Staff Years: .1

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. The clinical characteristics, radiologic, microbiologic, and cytopathologic results of pulmonary infections are available for a total of 50 patients. To date, the microbiologic and radiologic data have been catalogued. Over 600 microbiologic procedures and 1,800 radiologic procedures have been reviewed. At the completion of the project, a diagnostic, evidenced-based algorithm will be available to aid in the diagnosis of pulmonary infections in patients with CGD.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000171-07

LBC: CCM

Title: Inflammatory Responses to Bronchial Endotoxin Instillation in Humans

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Anthony F. Suffredini, MD (CCM, CC)

Collaborators, Lab: Steven Banks, PhD (CCM, CC)
Carmen Fiuza, MD (CCM, CC)
Debra G. Reda, RN (CCM, CC)
Margaret M. Tropea (CCM, CC)

Total Staff Years: 1.7

Human Research: Human cells or tissues

Keywords: Endotoxin, lung inflammation, innate immunity

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 CL000182-07

LBC: CCM

Title: Study of Control of Cytosolic Phospholipase A2 Activity

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James H. Shelhamer, MD (CCM, CC)

Collaborators, Lab: James Copeland, MD, PhD (CCM, CC)
Rafal Pawliczak, MD, PhD (CCM, CC)

Total Staff Years: 1.1

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 proteins (p11) was identified as interacting with cPLA2. The promoter region of the p11 gene has been cloned and is being characterized. Ongoing studies include production of recombinant protein and modulation of enzyme function by oxidant molecules. One manuscript is in press and another is in preparation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000188-05

LBC: CCM

Title: Reactive Oxygen Species Signaling by Endothelial Nitric Oxide Synthase

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Robert L. Danner, MD (CCM, CC)

Collaborators, Lab: Robert Ashe, BA (CCM, CC)
Ana del P. Cintron, MT (ASCP) (CCM, CC)
Xiaolin Cui, MD (CCM, CC)
Penglin Ma, MD (CCM, CC)
Shuibang Wang, MD (CCM, CC)

Total Staff Years: 1.8

Human Research: Human cells or tissues

Keywords: Nitric oxide synthases, nitric oxide

Summary: Nitric oxide synthases (NOS), 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 TNFα production was increased, but that this effect was not related to NO production. Further work found that eNOS upregulates TNFα by producing a reactive oxygen species (ROS) (*J Biol Chem*, 2000). Recent experiments have demonstrated that eNOS upregulation of TNFα 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 mechanism(s) 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 CL000189-05

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Henry Masur, MD (CCM, CC)

Collaborators, Lab: Joseph A. Kovacs, MD (CCM, CC)
Barbara K. Hahn (CCM, CC)

Collaborators, NIH: Henry Clifford Lane, MD (NIAID)
Steven H. Fischer, MD, PhD (DLM, CC)
Vee J. Gill, PhD (MICRO, CC)
Sheng-ning Huang (CCM, CC)
Jodie M. Parker (CC)

Collaborators, Extramural: Laurence Huang, MD (University of California – San Francisco)
Daniel Lucey, MD (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. During this trial, resulting studies have moved the field from a focus on tissue to a focus on respiratory secretions, especially secretions that can be obtained non-invasively. Samples for control patients are being collected as well. First, a polymerase chain reaction (PCR) technique using a unique major surface glycoprotein primer was assessed 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. A prospective trial evaluating the utility of quantitative PCR is almost completed in conjunction with the University of California – San Francisco.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000190-05

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Mark T. Gladwin, MD (CCM, CC)

Collaborators, NIH: Richard O. Cannon, MD (CB, NHLBI)
Alan N. Schechter, MD (LCB, NIDDK)
Constance T. Noguchi, PhD (MCB, LCB, NIDDK)
Griffin P. Rodgers, MD (MCHB, NIDDK)
James H. Shelhamer, MD (CCM, CC)

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.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000191-05

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Kovacs, MD (CCM, CC)

Collaborators, NIH:
Judith Falloon, MD (CMRS, NIAID)
Henry Clifford Lane, MD (CMRS, NIAID)
Henry Masur, MD (CCM, CC)
Michael Polis, MD (CMRS, NIAID)
Jorge L. Tavel, MD (DIR, NIAID)
Richard T. Davey, MD (CRS, NIAID)
Dimiter S. Dimitrov, PhD (NCI)
Grace G. Kelly, RN (CCM, CC)
Susan Leitman, MD (DTM, CC)
William R. Sachau (CRS, NIAID)
Douglas J. Schwartzentruber, MD (SB, NCI)
Igor A. Sidorov (NCI)

Collaborators, Extramural:
Michael Baseler, PhD (SAIC)
Joseph Aldesberger, PhD (SAIC)
Richard Lempicki, PhD (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) Bromodeoxyuridine (BrDU; 200 mg/m2), an analogue of thymidine, will be administered to HIV-infected patients. BrDU is incorporated into DNA, and incorporation can be measured using an anti-BrDU monoclonal antibody. By fluorescence-activated cell sorter (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, 50 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 the second 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. Over the past year we have examined lymphocyte kinetics in patients receiving IL-2, and have found that intermittent IL-2 therapy expands the numbers of both CD4 and CD8 cells, which then have a very long survival in responding patients. 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.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000192-05

LBC: CCM

Title: Molecular Studies of Human *Pneumocystis carinii*

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Kovacs, MD (CCM, CC)

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

Collaborators, NIH: Henry Masur, MD (CCM, CC)
Steven H. Fischer, MD, PhD (DLM, CC)
Vee J. Gill, PhD (MICRO, CC)

Collaborators, Extramural: Charles B. Beard, PhD (CDC)
Laurence Huang, MD (University of California – San Francisco)

Total Staff Years: .8

Human Research: Human cells or tissues

Keywords: *Pneumocystis carinii*

Summary: *Pneumocystis carinii* (*P. 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, non-invasive 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, since 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 and investigators at San Francisco General Hospital and the Centers for Disease Control and Prevention. 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 developed a rapid screening assay for agents that target DHFR by expressing the enzyme in a yeast system. We have also developed a rapid method for detection of DHPS mutations using single strand conformational polymorphisms (SSCP) and have examined a large number of samples for DHPS mutations, including organisms obtained from an Italian cohort. Over the past year we have developed and evaluated 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, MD

Collaborators, Lab: Steven Banks (CCM, CC)
Peter Q. Eichacker, MD (CCM, CC)
Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Stephen S. Richmond (CCM, CC)
Steven Solomon, PhD (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 CL001157-04

LBC: CCM

Title: Effects of Inhaled Nitric Oxide on Pulmonary Inflammatory Responses

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Anthony F. Suffredini, MD (CCM, CC)

Collaborators, Lab: Steven Banks, PhD (CCM, CC)
Carmen Fiuza, MD (CCM, CC)
Mark T. Gladwin, MD (CCM, CC)
Debra G. Reda, RN (CCM, CC)
Margaret M. Tropea (CCM, CC)

Total Staff Years: .9

Human Research: Human cells or tissues

Keywords: Nitric oxide, pulmonary inflammation, innate immunity

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 nitric oxide for acute lung injury. After 4 days of inhaled NO, patients had a reduction of BAL neutrophil spontaneous H_2O_2 production, CD11b/CD18 expression, and less 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 non-rebreathing 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.

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Robert L. Danner, MD (CCM, CC)

Collaborators, Lab: Robert Ashe, BA (CCM, CC)
Xiaolin Cui, MD (CCM, CC)
Penglin Ma, MD (CCM, CC)
Shuibang Wang, MD (CCM, CC)
Jianhua Zhang, PhD (CCM, CC)

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

Total Staff Years: 1.8

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 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 are being investigated. Work with the IL-8 promoter suggest 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 are being 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 CL001159-04

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Henry Masur, MD (CCM, CC)

Collaborators, Lab: Grace G. Kelly, RN (CCM, CC)
Joseph A. Kovacs, MD (CCM, CC)

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

Total Staff Years: .5

Human Research: Human subject research

Keywords: Magnetic resonance imaging, HIV

Summary: In 2002 we reported the occurrence of avascular necrosis (AVN) of bone (usually the hip) in 15 asymptomatic patients with HIV disease. We are currently assessing the natural history of avascular necrosis in this chart and in an additional ten patients with symptomatic disease. The occurrence of pain and functional disability is being assessed. The development of AVN at other sites is also being evaluated by yearly magnetic resonance imaging scans. The study will elucidate natural history and risk factors for this unexpected complication of treated HIV disease.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001160-04

LBC: CCM

Title: Study of the Control of p11 Protein Production

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James H. Shelhamer, MD (CCM, CC)

Collaborators, Lab: Xiuli Huang, MD (CCM, CC)
Rafal Pawliczak, MD, PhD (CCM, CC)

Total Staff Years: .9

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. p11 gene expression is enhanced by stimulation with epidermal growth factor or with interferon gamma. Two manuscripts have been published and two are in preparation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001163-03

LBC: CCM

Title: Endothelial Cell Response to Oxidative Stress

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James H. Shelhamer, MD (CCM, CC)

Collaborator, Lab: Uday Nanavaty, MD (CCM, CC)

Total Staff Years: .6

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. Oxidative stress induces necrosis in human lung epithelial cells. Similar levels of oxidative stress are not lethal to endothelial cells, which respond with contraction and with production of gene expression in a variety of antioxidant proteins. One manuscript is in press.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001164-03

LBC: CCM

Title: Functional Genomics of Critical Illness

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Robert L. Danner, MD (CC)

Collaborators, Lab: Robert Ashe, BA (CC)
Ana del P. Cintron, MT (ASCP) (CC)
Charles Natanson, MD (CC)
Zenaide Quezado MD (CC)
James H. Shelhamer, MD (CC)
Michael A. Solomon, MD (CC)
Anthony F. Suffredini, MD (CC)
Shuibang Wang, MD (CC)
Jianhua Zhang, PhD (CC)

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

Collaborators, Extramural: J. Perren Cobb, MD (Washington University)
Umberto Meduri, MD (University of Tennessee)

Total Staff Years: 3.2

Human Research: Human subject research: Human cells or tissues

Keywords: Critical illness, genomics

Summary: Critical illness syndromes – such as acute respiratory distress syndrome, septic shock, myocardial depression, and multiple organ failure – all share the hypothesis that the host response plays a central pathogenic role. In this project, Critical Care Medicine Department 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. Bacterial challenge in rats has shown that a number of genes are expressed in a graded manner that corresponds closely to infection severity.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001165-03

LBC:	CCM
Title:	Influence of Site, Severity, and Type of Infection on the Effects of Endotoxin Analogue in Sepsis
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Peter Q. Eichacker, MD (CCM, CC)
Collaborator, Lab:	Steven Solomon, PhD (CCM, CC)
Collaborators, Extramural:	Seiichi Kobayashi, PhD (Eisai Research Institute) Akiyoshi Suganuma, DVM, PhD (Easai Research Institute)
Total Staff Years:	.2
Human Research:	Neither human cells nor tissues
Keywords:	Sepsis, endotoxin analogue

Summary: Lipopolysaccharide (LPS) release from invading bacteria has been closely associated with the pathogenesis of the inflammatory tissue injury occurring during gram-negative sepsis in humans. Agents designed to inhibit endotoxin have been proposed as adjunctive therapy for sepsis. E5564, a lipid A analogue, is one such agent, which has been shown to competitively inhibit LPS-stimulated cytokine release from macrophages. However, although LPS signaling may stimulate inflammatory mediators harmful to the host, this response may also have an adaptive protective function. In fact, other agents (e.g., anti-endotoxin antibodies) designed to inhibit LPS that were beneficial in animal models of sepsis have not shown beneficial effects in large clinical sepsis trials. It is possible that in animal models that have frequently employed intravascular (IV) bacterial challenges, the effects of LPS on host defense and inflammatory injury are different than during the extravascular (EV) infection primarily observed in patients. For instance, the intravascular activation of leukocytes by endotoxin may have little protective effect during IV bacterial challenge but may be important for their recruitment to an EV nidus of infection. This study therefore compared the effects of E5564 with similarly lethal IV and EV infection. Rats received E5564 or placebo after IV or EV (intrabronchial or intraperitoneal) *E. coli* challenges. E5564 decreased the relative risk of death with IV *E. coli* and increased it with EV infection in patterns that were significantly different. Compared to controls, in both IV and EV *E. coli*, E5564 increased circulating total leukocytes and neutrophils at 4 and 24 h combined but decreased lung lavage neutrophils at 4 h while increasing them at 24 h. Thus, the ability of E5564 to impair tissue leukocyte recruitment may explain the lack of benefit or potential harm associated with the agent in this model of EV infection. Conversely, in IV infections, the same effect on leukocyte trafficking may limit nonspecific organ injury and thereby improve survival. Site of infection may have an important impact on agents designed to alter LPS levels in sepsis. 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 minimally affected 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 that competitively inhibit cell signaling by endotoxin. E5564 is one such competitive inhibitor.

LBC: CCM

Title: Gene Expression and Protein Profiles in Humans Challenged with Endotoxin

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Anthony F. Suffredini, MD (CCM, CC)

Collaborators, Lab: Ana del P. Cintron, MT (ASCP) (CCM, CC)
Robert L. Danner, MD (CCM, CC)
Carmen Fiuza, MD (CCM, CC)
Carolea Logun (CCM, CC)
Patricia J. Madara (CCM, CC)
Debra G. Reda, RN (CCM, CC)
James H. Shelhamer, MD (CCM, CC)
Shefali Talwar, MD (CCM, CC)
Margaret M. Tropea (CCM, CC)

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

Total Staff Years: 2.6

Human Research: Human cells or tissues

Keywords: Endotoxin, microarrays, functional genomics, proteomics, innate immunity

Summary: Reliable biomarkers are needed to identify patients with sepsis who will benefit from anti-inflammatory therapies. In addition, recent observations suggest that previously unrecognized 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 upon 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 that 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 cells and in separate studies, cells obtained with bronchoalveolar lavage from the lung. The temporal pattern of gene expression will be studied using cDNA oligonucleotide microarrays. In addition, plasma and bronchoalveolar lavage will be evaluated using 2-dimensional gel electrophoresis and protein chips (surface-enhanced laser desorption ionization arrays) to identify new proteins and their pattern of expression during this acute inflammatory response. These tools will be useful to study fundamental aspects of gene and protein expression during exposure to bacterial products. They will provide a means of characterizing new mediators and mechanisms that are part of the acute phase response to bacterial products.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001169-03

LBC: CCM

Title: Inflammatory Effects of High Mobility Group Protein 1

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Anthony F. Suffredini, MD (CCM, CC)

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

Collaborator, NIH: Michael Bustin, PhD (LMC, NCI)

Total Staff Years: 1.5

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-1 (HMGB1) is a non-histone DNA-binding protein that facilitates transcription. Recently, investigators have shown that HMGB1 has other roles that may be critical in the development of sepsis and septic shock. HMGB1 is released as a late mediator of sepsis (i.e., after 8 to 15 hours) from mononuclear cells stimulated with tumor necrosis factor, IL-1, or endotoxin. It is detected in the blood of septic mice and in septic patients and it worsens outcome when given to septic mice. It plays a role in migrating axons of neurons in the developing brain and it 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 HMGB1 to acute human inflammation, we are producing recombinant human HMGB1 in a bacterial expression system and are using the protein to study inflammatory responses in endothelium, respiratory epithelium, 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 their responses to HMGB1. HMGB1 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 HMGB1 on target cells. These data should provide important new information regarding the role of HMGB1 in acute human inflammation to bacterial products.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001170-02

LBC: CCM

Title: Global Initiative to Characterize Antiretroviral Pharmacokinetics

Dates: from 12/31/2001 to 12/31/2002

Principal Investigator: Scott R. Penzak, Pharm D (CC)

Supervisor of Record: Henry Masur, MD (CCM, CC)

Collaborator, NIH: Jorge L. Tavel, MD (DIR, NIAID)

Collaborator, Extramural: Peter Mugyenyi, MD (Joint Clinical Research Center, Butikiro House)

Total Staff Years: .34

Human Research: Human subject research

Keywords: HIV, developing world, pharmacokinetics, antiretroviral, generic, genetics, ethnicity, pharmacogenomics

Summary: The overwhelming majority of HIV-infected persons reside in the developing world. As such, recent efforts have focused on providing antiretroviral pharmacotherapy to this population. However, there are a number of factors indigenous to non-Western HIV-infected patients that may alter their virologic, immunologic, and/or toxicologic response to antiretroviral therapy. Absorption, distribution, and clearance of antiretroviral medications may differ among patients residing in non-Western countries secondary to dietary influences, parasitic infection, and malabsorption. Genetic polymorphisms of drug-metabolizing enzymes (cytochrome P450; CYP) and drug transporters (P-glycoprotein) as well as generic formulations of antiretroviral medications may also contribute to altered pharmacokinetics among these patients. The purpose of this pilot, hypothesis-generating study is (1) to characterize the pharmacokinetics of the non-nucleoside reverse transcriptase inhibitor nevirapine in a non-Western HIV-infected population (Kampala, Uganda) and in a similar cohort of HIV-infected individuals in the United States and (2) to compare pharmacokinetic parameter values between the groups. Twenty-five subjects from each site will participate. Subjects from the Ugandan site may participate regardless of their CD4+ lymphocyte count and viral load; they will be studied prior to the U.S. cohort. The U.S. group will be selected to include subjects who are demographically similar to their Ugandan counterparts. Subjects will have one pre-dose and two post-dose blood samples collected for the determination of nevirapine plasma concentrations. Samples will be analyzed using LC/MS-MS Population pharmacokinetic parameter values (Cmax, Cmin, AUC, CL/F, Vd) will be determined using NONMEM and compared between groups. Blood samples collected during the study may also be used to determine CYP and MDR1 genotypes of study subjects in an effort to explain any observed differences in pharmacokinetic parameter values between the study populations. In a first phase of ensuring comparability of products, tablets containing nevirapine (from six international sources representing three manufacturers) were assayed for drug content; all were found to contain the labeled amount of drug (200 mg).

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001171-02

LBC: CCM

Title: Expression Profiling in Acute Cardiac Allograft Rejection

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Michael A. Solomon, MD (CCM, CC)

Supervisor of Record: Robert L. Danner, MD (CCM, CC)

Collaborators, Lab: Robert Ashe, BA (CCM, CC)
Kenneth Dabbs (CCM, CC)
Sameena S. Khan, MD (CCM, CC)
Charles Natanson, MD (CCM, CC)
Rajnish Prasad, MD (CCM, CC)
Anthony F. Suffredini, MD (CCM, CC)
Jianhua Zhang, PhD (CCM, CC)

Collaborators, NIH: Peter J. Munson, PhD (ABS, CIT)
Paul Hwang, MD (NHLBI)
J. Philip McCoy (NHLBI)

Collaborators, Extramural: Nelson Burton, MD (Cardiovascular Surgery, INOVA Fairfax)
Andrew J. Keller, MD (Heart Transplant Program, INOVA Transplant Center)

Total Staff Years: 2.28

Human Research: 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 noninvasive method exists for the diagnosis of acute cardiac rejection. Several methodologies have been studied, including electrocardiography, echocardiography, nuclear imaging, and phosphorus spectroscopy, without success. 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 CL001172-02

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigators: Steven Solomon, PhD (CCM, CC)
Charles Natanson, MD (CCM, CC)

Collaborators, Lab: Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Steve Richmond (CCM, CC)

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

Total Staff Years: 1

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 remains unknown. Proinflammatory 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 proinflammatory response disrupt the integrity of the myocardial microvasculature and subsequently injure the myocytes, resulting in myocardial functional depression. Similar to 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 left ventricular (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 proinflammatory 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 CL001173-02

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Mark T. Gladwin, MD (CCM, CC)

Collaborators, Lab: Maria L. Jison, MD (CCM, CC)
James S. Nichols, RN (CCM, CC)

Collaborators, NIH: R.O. Cannon, MD (NHLBI)
Griffin P. Rodgers, MD (MCHB, NIDDK)
Vandana Y. Sachdev (CB, NHLBI)
Alan N. Schechter, MD (LCB, NIDDK)

Total Staff Years: .3

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 to determine 1) the pathophysiologic processes that are associated with and potentially contribute to secondary pulmonary hypertension in adult patients with sickle cell anemia, 2) 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) 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 CL001174-02

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Mark T. Gladwin, MD (CCM, CC)

Collaborators, Lab: Maria L. Jison, MD (CCM, CC)
James S. Nichols, RN (CCM, CC)

Collaborators, NIH: Richard O. Cannon, MD (CB, NHLBI)
Vandana Y. Sachdev (CB, NHLBI)
Alan N. Schechter, MD (LCB, NIDDK)

Total Staff Years: .3

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 to 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 determine the prevalence and prognosis of secondary pulmonary hypertension in adult patients with sickle cell anemia and to determine whether genetic polymorphisms in candidate genes contribute to its development.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001175-02

LBC: CCM

Title: Delivery of Nitric Oxide by Hemoglobin to Improve Blood Flow in Sickle Cell Disease

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Mark T. Gladwin, MD (CCM, CC)

Collaborators, Lab: Robert L. Danner, MD (CCM, CC)
James S. Nichols, RN (CCM, CC)

Collaborators, NIH: Richard O. Cannon, MD (CB, NHLBI)
Alan N. Schechter, MD (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 CL001178-02

LBC: CCM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Q. Eichacker, MD (CCM, CC)

Collaborator, Lab: Xizhong Cui (CCM, CC)

Collaborator, Extramural: Daniela Salvemini (Metaphor Corp.)

Total Staff Years: .35

Human Research: Neither human cells nor tissues

Keywords: Sepsis, treatment, superoxide, superoxide dismutase mimetic

Summary: Superoxide anion production is necessary for leukocyte, vascular endothelial, and other functions during infection. However, excessive production of superoxide and its reactant products has been implicated in the pathogenesis of tissue injury and organ dysfunction occurring during sepsis and septic shock. Examination of tissue samples in animal models has also suggested that depletion of endogenous antioxidants such as superoxide dismutase during sepsis may potentiate this injury. As a result, antioxidant treatments employing low molecular weight nonprotein membrane-permeable superoxide dismutase mimetics have been developed for use in sepsis and other conditions associated with increased systemic inflammation. These agents, which are metal-chelated macrocyclic ligand complexes, demonstrate free radical scavenging activities similar to superoxide dismutase. M40401 and M40403 are two such agents which show novel selectivity for superoxide anion itself. Superoxide anion, however, may have divergent effects during sepsis. In addition to the opposing roles superoxide might have on microbial killing and secondary inflammatory tissue injury, it has the potential to alter hemodynamic function in opposing ways. Excessive superoxide production has been implicated in the oxidation of both endogenous and exogenous catecholamines which normally cause vasoconstriction. On the other hand, superoxide anion contributes to the inactivation of nitric oxide, a potent vasodilator. As a result, the overall effects of superoxide anion on vascular tone may represent its relative contribution to the inactivation of catecholamines versus nitric oxide. These contributions may vary during sepsis dependent on its severity. In turn, the hemodynamics effects of superoxide inhibitors like M40401 may also be influenced by the underlying severity of sepsis. The present studies investigated whether the severity of infectious challenge and its associated risk of death would alter the efficacy of M40401 in a rat model of sepsis. In individual experiments, animals were randomized to be challenged with doses of intravenous *E. coli* designed to produce low or high control mortality rates, following which they were treated with M40401 or placebo. The results showed that the efficacy of M40401 was dependent on control mortality rates. In experiments with high control mortality rates (i.e., > median), M40401 increased survival rates and mean arterial blood pressure and decreased platelet counts. However, in experiments with low control mortality rates (i.e., (i.e., <median), M40401 had opposite effects.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001179-01

LBC:	CCM
Title:	Differentiation of Acute Rejection from Infection in a Rat Heart Transplant Model
Dates:	from 07/18/2002 to 09/30/2002
Principal Investigator:	Michael A. Solomon, MD (CCM, CC)
Supervisor of Record:	Robert L. Danner, MD (CC)
Collaborators, Lab:	Mark D. Miller (CC) Steven Solomon, PhD (CCM, CC) Robert Ashe, BA (CCM, CC) Xizhong Cui (CCM, CC) Katherine J. Deans, MD (CCM, CC) Melinda S. Fernandez (CCM, CC) Yvonne C. Fitz (CCM, CC) Allen T. Hilton (CCM, CC) Peter C. Minneci, MD (CCM, CC) Shuibang Wang, MD (CCM, CC) Jianhua Zhang, PhD (CCM, CC)
Collaborators, NIH:	Peter Q. Eichacker, MD (CCM, CC) Monica Bur (OD) Tanya Burkholder, DVM (OD) Adrienne Hergen (OD) Kathryn Hope (OD) Katherine Lucas (OD) Chris Romines, DVM (OD) Tom (Marvin) Thomas, DVM (OD)
Collaborator, Extramural:	Rosaly Correa, MD, PhD (Center for Practice and Technology Assessment)
Total Staff Years:	2.6
Human Research:	Neither human cells nor tissues
Keywords:	Rodent, transplant, heart, lymphocyte, rejection, infection, DNA microarray

Summary: Acute cardiac allograft rejection and infection remain significant sources of morbidity and mortality after heart transplantation, accounting for nearly 50 percent of reported deaths. It is often difficult to clinically distinguish between rejection and infection because they are both inflammatory processes with similar, nonspecific symptoms. However, this differential is essential for determining therapy. Identifying laboratory methods that will permit safe and concise early differentiation between rejection and infection in the transplant patient will improve outcome substantially. The primary goal of this study is to determine whether gene microarray analysis of peripheral blood mononuclear cells (PBMCs) will reliably differentiate acute heart rejection from infection in the transplanted rat. Our protocol will combine two well-established

rat models, a heterotopic heart transplantation model and an *E. coli* pulmonary infection model. The study is divided into two stages In the first stage, cardiac transplant techniques in the rat will be refined and the optimal doses and duration of both cyclosporin (CSA) therapy and *E. coli* bacterial infection will be determined. In our first pilot study, we will establish a dose and duration of CSA therapy in this model that reliably suppresses rejection during its administration, but will permit the emergence of rejection upon its discontinuation. Our second pilot study will establish a dose and duration of intrabronchial *E. coli* pneumonia that is sufficient to cause a systemic inflammatory response without being immediately lethal in transplanted rats receiving CSA. The second stage of our study will determine whether gene microarray analysis of PBMCs is capable of differentiating cardiac rejection from infection. In this stage all rats will undergo heart transplantation on day zero in conjunction with daily CSA (dose determined in stage 1) to suppress rejection. After transplant, animals will be randomized (timing determined in stage 1) to have CSA discontinued, in order to initiate rejection, or continued, in order to further suppress rejection. After discontinuing CSA the animals will again be randomized (timing determined in stage 1) to receive intrabronchial *E. coli* inoculation or saline inoculation. Consequently, four groups will be studied: No rejection (receiving CSA) without infection, no rejection (receiving CSA) with infection, rejection (not receiving CSA) without infection, and rejection (not receiving CSA) with infection. At the end of the study, all animals will be sacrificed and the blood and heart removed for gene microarray analysis. Other analytic tools that may be employed include RT-PCR, Western blot, *in situ* hybridization, proteomics, immunohistochemistry, and histopathology. In addition, the animals' lungs, spleen, and liver will be procured in the primary study and preserved for potential future analysis.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001180-01

LBC: CCM

Title: Effect of Intra-aortic Balloon Pump in a Canine Model of Septic Shock

Dates: from 08/21/2001 to 09/30/2002

Principal Investigator: Michael A. Solomon, MD (CCM, CC)

Supervisor of Record: Charles Natanson, MD (CCM, CC)

Collaborators, Lab: Steven Solomon, PhD (CCM, CC)
Katherine J. Deans, MD (CCM, CC)
Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Peter C. Minneci, MD (CCM, CC)
Stephen S. Richmond (CCM, CC)

Collaborators, NIH: John D. Bacher, BS, DVM, MS (SRU, SSB, OD)
Monica Bur (OD)
Tanya Burkholder, DVM (OD)
Adrienne Hergen (OD)
Kathryn Hope (OD)
Katherine Lucas (OD)
Kelli Matisak (OD)
Mark D. Miller (CC)
Chris Romines, DVM (OD)
Tom (Marvin) Thomas, DVM (OD)

Total Staff Years: 2.3

Human Research: Neither human cells nor tissues

Keywords: Animal model, sepsis, intra-aortic balloon pump, hemodynamics, vasopressor

Summary: Septic shock is the most common cause of death in medical and surgical intensive care units in the United States. Thirty percent of patients who die from sepsis are noted to have low cardiac output. The purpose of this study is to examine the role of intra-aortic balloon pump counterpulsation (IABC) in the treatment of septic shock. The goal of placing an intra-aortic balloon pump (IABP) is twofold. It reduces the afterload on the heart, thereby allowing it to do less work (assisted systole), while enhancing its coronary blood flow, thereby providing it with more energy (diastolic augmentation). This is the first controlled, randomized survival study of IABC in a well-characterized low cardiac output animal model of sepsis. This study has three phases: phase 1 (baseline), phase 2 (sepsis), and phase 3 (recovery). During the sepsis phase all animals will receive bacterial clot (18 x 109 cfus of *E. coli*), intravenous fluids (Ringer's solution with 5 percent dextrose), and antibiotics (Ceftriaxone). The animals will be randomized to one of four groups: Group 1 (control group), Group 2 (vasopressors), Group 3 (IABP), and Group 4 (vasopressors + IABP). This study design will allow us to determine the benefit of each treatment intervention (vasopressors or IABP) compared with control and to detect any interaction between the interventions. The same animals will be studied in each of the three

phases. The measurements to be obtained in each phase of the study include hemodynamics (blood pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, ejection fraction, and heart rate), venous and arterial blood gases, complete blood counts, serum chemistries, quantitative blood cultures, endotoxin levels, tumor necrosis factor levels, lactate levels, creatinine kinase levels, and creatinine kinase isoenzymes-CK and CK-MM. After study measurements have been obtained, all catheters will be removed from the animals and they will be returned to their cages. The overall effect of the IABC is to increase myocardial oxygen supply by increasing coronary perfusion during diastole and decrease myocardial oxygen demand by decreasing afterload during systole. We believe that the IABC may reduce the myocardial depression of sepsis by improving coronary blood flow and reducing left ventricular work, therefore resulting in a decrease in mortality during sepsis.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001181-01

LBC: CCM

Title: Effect of Vasopressin and Norepinephrine in a Canine Model of Septic Shock

Dates: from 10/01/2002 to 09/30/2002

Principal Investigators: Steven Solomon, PhD (CCM, CC)
Charles Natanson, MD (CCM, CC)

Collaborators, Lab: Katherine J. Deans, MD (CCM, CC)
Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Peter C. Minneci, MD (CCM, CC)
Stephen Richmond (CCM, CC)

Total Staff Years: 3.1

Human Research: Neither human cells nor tissues

Keywords: Sepsis, vasopressors, vasopressin, norepinephrine

Summary: The purpose of this clinical study is to examine the roles of vasopressin and norepinephrine in the treatment of septic shock and their impact on survival. Norepinephrine, a vasopressor, is the most commonly used clinical agent to reverse the lethal hypotension in patients with septic shock. A new therapy for sepsis has been the use of vasopressin to treat the hypotension associated with sepsis. Small studies have examined vasopressin in conjunction with norepinephrine and suggest that their use together may lower norepinephrine requirements in septic patients. High doses of either vasopressin or norepinephrine can lead to negative outcomes, including decreased end organ perfusion and decreased cardiac output. It is believed that by combining these two drugs, there will be less vasoconstriction-related organ injury and an increased benefit in survival rate. However, no clinical or animal study has examined the effect of norepinephrine, vasopressin, or a combination on survival rate, blood pressure, and organ injury. This is, in part, due to the lethality of the disease, where withholding such therapies would be impossible. Of particular concern is that it is entirely possible that some of these vasopressors can improve blood pressure but adversely affect survival. L-NMMA, a vasopressor, increased blood pressure but worsened outcome in this model of sepsis. Unfortunately, these results were confirmed in humans with septic shock. Previously, in a pilot study, we determined doses of each drug used alone that raised blood pressure but did not adversely affect survival rate. This study will examine the role of vasopressin and norepinephrine used alone and together in treating sepsis in the canine model. For the first time in any model of sepsis we will determine the effects of vasopressin and norepinephrine on blood pressure, cardiac output, and survival.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001182-01

LBC: CCM

Title: Early Detection of Arrhythmogenic Right Ventricular Cardiomyopathy

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Dorothea McAreavey, MD (CCM, CC)

Supervisor of Record: James H. Shelhamer, MD (CCM, CC)

Collaborators, Lab: Naomi P. O'Grady, MD (CCM, CC)
Rose M. Wienhoff, CRNP (CCM, CC)

Collaborators, NIH: Andrew E. Arai, MD (LCE, NHLBI)
Lameh Fananapazir, MD (NHLBI)
Eric Leifer, PhD (NHLBI)
Saidi A. Mohiddin, MB, ChB (CB, NHLBI)
Vandana Y. Sachdev (CB, NHLBI)

Collaborators, Extramural: Milan Horacek, PhD (Dalhousie University)
Jeffrey P. Moak, MD, FACC (Cardiology Department, Children's National Medical Center)
Renu Virmani, MD (AFIP)

Total Staff Years: 1.1

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

Keywords: Arrhythmogenic right ventricular vardiomyopathy, sudden death, early detection of disease

Summary: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a familial heterogeneous clinical and molecular disease characterized by dilatation and dysfunction of the right ventricle and ventricular arrhythmias. The ventricular arrhythmias are heart rate and catecholamine dependent. There may also be involvement of the left ventricle. The diagnosis of ARVC is critical because therapy, including implantable defibrillators, may prevent sudden death. However, identification of subjects at risk remains a major challenge due to limitations of imaging and diagnostic techniques. The proposed study is designed to investigate subjects at risk for ARVC because of a positive family history. Studies will include magnetic resonance imaging, investigation of the utility of a novel diagnostic test, and genetic studies. A protocol has been submitted to the Institutional Review Board.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001183-01

LBC: CCM

Title: Interferon Induction of Gene Expression in Human Lung Epithelial Cells

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James H. Shelhamer, MD (CCM, CC)

Collaborators, Lab: Carolea Logun (CCM, CC)
Rafal Pawliczak, MD, PhD (CCM, CC)

Total Staff Years: 1.1

Human Research: Human cells or tissues

Keywords: Cytokines, inflammation, cell responses

Summary: The effect of interferon gamma gene expression in human lung epithelial cells was studied in primary cultures of human epithelial cells. Functional genomic studies were carried out using an Affymetrix platform. Over 600 genes were found to be up- or down-regulated at 24 hours. Co-treatment with dexamethazone inhibited expression of a variety of inflammatory and cell cycle genes induced by interferon gamma. A manuscript is in preparation.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001184-01

LBC: CCM

Title: Effect of Epinephrine in a Canine Model of Septic Shock

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Steven Solomon, PhD (CCM, CC)

Supervisor of Record: Charles Natanson, MD (CCM, CC)

Collaborators, Lab: Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Peter C. Minneci, MD (CCM, CC)
Stephen Richmond (CCM, CC)

Total Staff Years: 1.7

Human Research: Neither human cells nor tissues

Keywords: Epinephrine, sepsis, septic shock, vasopressors

Summary: Early in the development of sepsis, the combined cardiopulmonary and peripheral effects result in decreased blood pressure, reduced ability to maintain blood pressure in the periphery, and normal to increased blood pumped out of the heart. In non-survivors, end-stage septic shock is characterized by low blood pressure and a reduced ability to pump blood out of the heart, resulting in multiple organ system failure and death. The inability to resolve the heart dysfunction and maintain adequate blood flow to the organs is the most significant contributing factor to the demise of the patient. Treatment of patients with sepsis consists of giving fluids and vasopressors to maintain blood pressure while giving antibiotics to kill the bacteria. At present, the canine model of sepsis uses a bacterial clot and is treated with fluids and antibiotics. The purpose of this study is to determine the number of animals necessary to determine the dose of epinephrine that will maintain blood pressure during sepsis and if this will have a beneficial effect on outcome. The use of epinephrine should allow us to more accurately characterize the treatment of sepsis in humans and address the low blood pressure and cardiac output.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001185-01

LBC: CCM

Title: Effect of Sympathetic Blockade in Non-lethal Sepsis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Steven Solomon, PhD (CCM, CC)

Supervisor of Record: Charles Natanson, MD (CCM, CC)

Collaborators, Lab: Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Peter C. Minneci, MD (CCM, CC)
Steve Richmond (CCM, CC)

Total Staff Years: 1.2

Human Research: Neither human cells nor tissues

Keywords: Epidural, morphine, bupivicaine, pain, sepsis

Summary: We investigated in a well-established canine model of human sepsis the effects of two different techniques of sympathetic blockade during bacterial peritonitis on pain relief, hemodynamics, and survival rate. Twenty-two purpose-bred beagles (12-28 months, 10-12 kg) were studied. Fourteen animals received an epidural infusion of bupivicaine and morphine, and the other 8 either a celiac plexus block (n = 4) or a sham block (n = 4). Eighteen of the 22 animals received an intraperitoneal challenge of *E. coli* (1- 10 x 10^9 colony forming units (cfus)/kg-1 bw) At comparable doses of intraperitoneal implanted *E. coli* (2.5-5 x 10^9 cfus/kg-1 bw), the addition of sympathetic blockade produced a synergistic decrease in survival times ($p = 0.002$) and mean left ventricular ejection fraction ($p = 0.008$), and an increase in creatinine levels ($p = 0.02$). There was also a significant increase in tumor necrosis factor levels ($p = 0.004$) and a decrease in blood endotoxin clearance ($p = 0.006$) associated with sympathetic blockade during sepsis. The celiac plexus-blocked animals had no improvement in pain scores and subjectively looked clinically worse than septic animals without a celiac plexus block. In contrast, the epidural block was effective in blocking the pain and discomfort associated with low-lethality doses of intraperitoneal bacteria, reflected by no increase in pain scores compared to animals not receiving bacterial challenge. This study shows that, during severe bacterial peritonitis, maintenance of sympathetic tone irrespective of pain relief provided is necessary for clearance of bacterial toxins, control of proinflammatory mediator release, hemodynamic stability, and survival. This study has presented two areas of further research: (1) to understand the relationship between sympathetic tone and control of bacterial clearance and proinflammatory mediator release, and (2) whether pain relief can be modulated to be effective without negatively affecting survival.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001186-01

LBC: CCM

Title: An Anthrax Lethal Toxin Model of Sepsis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Q. Eichacker, MD (CCM, CC)

Collaborator, Lab: Xizhong Cui (CCM, CC)

Collaborators, NIH: Stephen H. Leppla, PhD (OIIB, NIDCR)
Mahtab Moayeri, PhD (OIIB, NIDCR)

Total Staff Years: .35

Human Research: Neither human cells nor tissues

Keywords: Anthrax, sepsis, model, pathogenesis, treatment

Summary: Inhaled anthrax infection is a major bioterrorism threat today. Models that simulate this disease for the study of pathogenesis and treatment are needed. Anthrax infection begins as a local collection of alveolar spores, which then spread as invasive bacteria to the mediastinal structures. From there the infection is disseminated systemically by intravascular spread. Intravascular spread results in an increasing toxin release that contributes directly to death. Anthrax bacilli produce two different virulence factors: a polyglutamate capsule and a three-component exotoxin. The capsule resists phagocytosis while the toxin is capable of injuring and killing the cells, which it binds to. Macrophage killing by the toxin is important in the spread of the disease. The infection is described as a toxigenic one with most of its pathogenesis relating directly to the toxin or to the toxin's influence on potentially harmful host mediators. Therefore, animal models based on the toxin alone are capable of simulating many of the key pathogenic events associated with the infection itself. This is important because the toxin can be manipulated far more safely than the bacteria itself. All small animal models to date using toxin challenge have employed a single rapid bolus. Death in these models is relatively rapid, extending from 1 to 3 hours after challenge depending on the dose of toxin. However, such a challenge is not consistent with the natural course of this infection, which likely includes a gradual increase in the amount of toxin the host is dealing with. Such increases are reflected in the changes in blood bacteria concentrations that have been observed over time. Thus, an animal model simulating this progressive increase in toxin would better simulate conditions encountered clinically. This in turn would provide a more accurate assessment of evolving pathogenic events associated with toxin and, more important, would provide a better model to test the influence of therapies directed at inhibiting the toxin or its effects. The research underway for this project has so far shown that anthrax toxin administered as a 24-hour infusion in Sprague-Dawley rats produces a prolonged and significantly different time course in lethality compared with a similar weight-based bolus dose. This prolonged time course has permitted a more accurate assessment of the cardiopulmonary injury and cellular host response occurring in the model. In marked contrast to similarly lethal endotoxin models of sepsis, serum nitrate and nitrite levels were not increased either early (6 hours) or late (24 hours) following anthrax\toxin. Cytokine panels are now under analysis to better define the host inflammatory

response to lethal anthrax toxin challenge in the model. In controlled experiments we have now completed, Sprague-Dawley rats show the same pattern of response to toxin as Fischer rats, making our model even more applicable for laboratories elsewhere. Given the central role the macrophage is believed to have in the pathogenesis of anthrax infection, as part of this project, experiments are now underway to investigate the influence of granulocyte-macrophage colony stimulating factor treatment during prolonged toxin infusion.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001187-01

LBC: CCM

Title: Effect of Graded Levels of Infection on Gene Expression in a Rat Model of Sepsis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Q. Eichacker, MD (CCM, CC)

Collaborators, Lab: Xizhong Cui (CCM, CC)
Robert L. Danner, MD (CCM, CC)
Charles Natanson, MD (CCM, CC)

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

Total Staff Years: .55

Human Research: Neither human cells nor tissues

Keywords: Oligonucleotide microarray, sepsis, pathogenesis, treatment

Summary: Bacterial infection with sepsis is associated with a high mortality rate (29 percent). Identifying the mediators produced during sepsis that result in harmful effects or recovery, as well as the patterns of gene expression which control their production, would improve the development and administration of medicines designed to modulate this inflammatory response. However, the septic response involves the activation of several different proinflammatory plasma proteolytic cascades as well as the cellular production of proinflammatory molecular mediators (e.g., cytokines, adhesion molecules, growth factors, oxidants, nitric oxide). In addition to the release of proinflammatory mediators, this response is associated with the production of anti-inflammatory molecules that provide endogenous control over the response. Attempts to fully characterize this response in individual patients have been unsuccessful due in part to its complexity and redundant nature as well as to the disparate roles it plays both in host defense and tissue injury. Such a characterization may be essential, however, for the application of new therapies designed to modulate inflammation during sepsis in the future. Oligonucleotide microarray analysis is a rapidly growing technology that identifies individual or groups of genes that are up- or down-regulated in a sample of cells. Expression profiling data can be used to identify, on a genome-wide basis, the specific genes that are responsive to particular regulatory mechanism during the development of disease. A rat genome U34A array, which analyzes about 7,000 full-length sequences and approximately 1,000 EST clusters (GeneChip, Affymetrix, Inc., Santa Clara, CA) to measure rat gene expression has been produced. The primary purpose of the studies underway in this project is to apply oligonucleotide microarray to determine genes that may be important in the development of sepsis and septic shock during infection. Rat genome U34A arrays are being used to identify genes from circulating mononuclear cells (lymphocytes and monocytes) that are either up- or downregulated in dose-ordered fashion during graded bacterial infection either acutely, subacutely, or at recovery (6, 24, or 168 hours after the onset of infection, respectively) in a well-characterized rat model of sepsis. These genes or their gene products will then serve as targets for potential treatments or prognostic testing in later sepsis studies. Experiments thus far

completed have shown that there is a subpopulation of genes represented on the micro-arrays under study that do show significant dose-ordered expression levels. However, up- or down-regulation with infection which is apparent quickly at 6 hours does not demonstrate dose ordering until 24 hours. Furthermore, the majority of genes showing this pattern, rather than being up-regulated, are down-regulated. The relevance of these generalized expression patterns is now being analyzed for functionally related groups of genes as well as for individual genes producing products strongly associated with either the harmful or beneficial effects of the inflammatory response.

LBC: CCM

Title: Nitric Oxide Inhibition with DTPA/Fe3 in a Rat Model of Sepsis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Q. Eichacker, MD (CCM, CC)

Collaborator, Lab: Xizhong Cui (CCM, CC)

Collaborator, Extramural: Luis Molina (Molicorp)

Total Staff Years: .35

Human Research: Neither human cells nor tissues

Keywords: DTPA, nitric oxide, sepsis, pathogenesis, treatment

Summary: Excessive nitric oxide (NO) production has been closely associated with the hemodynamic instability and death occurring during sepsis and septic shock. Despite this, agents designed to inhibit the inducible form of NO synthase (NOS), while increasing blood pressure, worsened outcome in patients with sepsis. Alternative methods for inhibiting the potentially harmful effects of NO are therefore now under study. One such agent is diethyltriaminepentacetate (DTPA) Iron III. This agent is a low molecular weight scavenger of free NO that does directly alter NOS function. Administration of DTPA Iron III in baboons challenged with a highly lethal dose of intravenous *E. coli* reduced intravascular nitrate levels and improved survival but did not have observable effects on hemodynamics. We studied whether this same agent would have similar beneficial effects in an animal model employing an extravascular site of infection. Rats were challenged with doses of *E. coli* via either intrabronchial or intravascular routes designed to produce high lethality rates. They were then treated with DTPA Iron III over a range of doses or placebo. Blood pressure, heart rate, and circulating cellular mediators were measured continuously for 24 hours and survival was observed for 168 hours. As would be expected based on its scavenging of NO, increasing doses of DTPA Iron III resulted in dose-ordered increases in blood pressure. However, no dose of DTPA Iron III with either intrabronchial or intravascular *E. coli* improved survival rates, and in most cases survival rates were reduced. Thus, in this rat model of sepsis, DTPA Iron III had very similar effects to those observed with NOS inhibitors in patients with sepsis. These results emphasize the important protective roles NO may play either in host defense or other functions during infection and sepsis.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001189-01

LBC: CCM

Title: The Influence of Infection Duration on TNF Ab in Sepsis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Q. Eichacker, MD (CCM, CC)

Collaborator, Lab: Steven Solomon, PhD (CCM, CC)

Total Staff Years: .2

Human Research: Neither human cells nor tissues

Keywords: Sepsis, model, pathogenesis, treatment

Summary: We showed previously that anti-inflammatory agents have differing effects in sepsis related to the underlying severity of infection. These agents were highly beneficial with severe sepsis when the risk of death was high, but they were less beneficial and potentially harmful with less severe infection. In these experiments, severity of infection was altered by varying the dose of infecting bacteria. However, the severity of infection in patients may relate not only to the number of bacteria they are initially exposed to but also to the time at which they present and begin antibiotic and supportive fluid treatment. In the present set of experiments, the influence of severity of infection on the anti-inflammatory agent tumor necrosis factor antibody (TNF Ab) will be studied. In these studies, however, severity of infection will be altered by varying the time at which animals receive antibiotic and fluid treatment following inoculation with similar doses of bacteria. Prior to the actual study of TNF Ab, however, a clinically relevant mouse model of sepsis required development, showing that the addition of fluid and antibiotic treatment would have beneficial effects as they are believed to have clinically. Experiments have been completed comparing the effects of antibiotics alone, fluids alone, antibiotics plus fluids, or no treatment in animals randomized to receive one of several increasing doses of intraperitoneal *E. coli* designed to produce low or high lethality rates. In a surprising finding that likely has clinical relevance, we have found that although fluid therapy alone has little beneficial effect, it synergistically increases the beneficial effects of antibiotics. In contrast to our prior experiments with anti-inflammatory agents, antibiotics either with or without fluids increased survival rates independent of the lethality of infectious challenge. Furthermore, delaying treatment with antibiotics and fluids resulted in time-ordered decreases in survival rates despite inoculation with similar doses of bacteria. This model will now allow us to evaluate the effects of varying treatment time with conventional sepsis therapies on the effects of anti-inflammatory agents like TNF Ab.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL008046-02

LBC: CCM

Title: The Role of Autonomic Innervation in the Interactions of Opioids and β-agonists

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Steven Solomon, PhD (CCM, CC)

Supervisor of Record: Charles Natanson, MD (CCM, CC)

Collaborators, Lab: Melinda S. Fernandez (CCM, CC)
Allen T. Hilton (CCM, CC)
Stephen Richmond (CCM, CC)

Total Staff Years: 1

Human Research: Neither human cells nor tissues

Keywords: Fentanyl, morphine, vagotomy, isoproterenol, halothane

Summary: Patients in the operating room or intensive care unit frequently need medications to improve the pumping ability of their hearts and their circulatory function. Commonly used medications for these purposes stimulate receptors in the cardiovascular system to achieve these results. These receptors are known as adrenergic receptors and are of two major types—alpha and beta. Alpha receptor stimulation causes arteries to constrict and thereby increases blood pressure. Beta receptor stimulation increases heart rate and pumping force and also dilates arterial blood vessels. Patients in the operating room and intensive care unit also receive narcotic medications like fentanyl as part of their anesthesia or for the treatment of pain. Fentanyl by itself has very little, if any, depressant effect on the heart except to decrease the heart rate. While this lack of cardiac effects has been described in the scientific literature, no studies have investigated whether fentanyl could decrease the effect of alpha- or beta-adrenergic stimulants on the cardiovascular system. In this study, we have shown in dogs that fentanyl diminishes the effectiveness of isoproterenol on cardiac function primarily through heart rate. We have also shown that the interaction between fentanyl and alpha and beta receptors is mediated by G-proteins. Studies are presently being performed to determine the neural role of this interaction. Additional studies investigating the role of heart rate by knocking out the sinoatrial node and controlling heart rate via pacing are also being conducted. Further understanding whether narcotics interact with cardiac stimulatory drugs within the heart could lead to better management of patients with shock or cardiac disease in the operating room or intensive care unit, and thereby improve outcome and potentially save lives.

DIAGNOSTIC RADIOLOGY DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL000077-05

LBC: DRD

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Richard Chang, MD (DDR, CC)

Supervisor of Record: King C. Li, MD (DDR, CC)

Collaborators, Lab: Clara Chen (DDR, CC)
Thomas Shawker, MD (DDR, CC)
Bradford J. Wood, MD (DDR, CC)

Collaborators, NIH: Mcdonald K. Horne, III (HEME, CC)
Richard O. Cannon, MD (CB, NHLBI)

Total Staff Years: 3

Human Research: Human subject research

Keywords: Deep vein thrombosis, thrombosis, thrombolytic therapy, recombinant tissue plasminogen activator

Summary: The objective of the study is to evaluate thrombolytic therapy using recombinant tissue plasminogen activator (rtPA) for treatment of acute deep vein thrombosis of the lower extremity. While the conventional therapy, anticoagulation alone, is highly effective in prevention of life threatening pulmonary embolism, it does not preserve venous function in the affected leg, often leading to postphlebitic syndromes in subsequent years. The study is designed to evaluate efficacy, safety, and cost of this form of treatment for restoration of venous function in the lower extremity. Since start of the study, 14 patients have been treated. All except one patient had significant improvement. Only two patients have had evidence of small pulmonary emboli during treatment, detected on ventilation perfusion lung scans that are obtained in 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 patients have required blood transfusions and no other complications have occurred.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000080-04

LBC:	DRD
Title:	Sonographic Evaluation of the Effects of Raloxifene on the Uterus and Ovaries in Premenopausal Patients
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Ahalya Premkumar (DDR, CC)
Supervisor of Record:	King C. Li, MD (DDR, CC)
Collaborators, Lab:	Nilo Avila, MD (DDR, CC) Diane A. Johnson (DDR, CC)
Collaborators, NIH:	Allison Baumann (NCI) Jennifer Eng-Wong, MD (NCI) Beth Schmidt (NCI) Pamela Stratton, MD (DIR, NICHD) David J. Venzon, PhD (BDMS, NCI) JoAnne Zujewski, MD (NCI)
Collaborators, Extramural:	Anna Parsons, MD (Department of Reproductive Ultrasound, University of South Florida)
Total Staff Years:	.9
Human Research:	Human subject research
Keywords:	Raloxifene, premenopausal, 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 is available regarding the gynecological effects of raloxifene in premenopausal women. The purpose of our study is to study both the short-term and the long-term effects of raloxifene on ovulation frequency, endometrial development, and cyclic function in general. The study started enrolling patients in January 1999. To date, 15 patients have been enrolled. One subject was dropped due to irregular menstrual cycles which made her ineligible. Two additional subjects dropped off from the protocol due to inability to comply with multiple study evaluations for logistic reasons. As the parent protocol (98-CC-0123) has completed accrual, no more patients will be enrolled into this protocol either. The protocol accrual therefore has been completed but we have not yet answered the research objectives.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000082-04

LBC: DRD

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Choyke, MD (DDR, CC)

Supervisor of Record: King C. Li, MD (DDR, CC)

Collaborators, Lab: Vincent B. Ho, MD (DDR, CC)
Ronald M. Summers, MD, PhD (DDR, CC)
Yantian J. Zhang (DDR, CC)

Collaborators, Extramural: Thomas Foo, PhD (General Electric Medical Systems)
Behram Pastakia, MD (Radiology, Washington VA Medical Center)
Azita Moalemi, MD (Cardiology, Mt. Vernon Cardiology)
Conor Lundergan, MD (Department of Cardiology, George Washington Medical Center)
Bradley Dick, MD (Chief, Interventional Radiology, Suburban Hospital)

Total Staff Years: .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 29 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 too preliminary but very promising. Real time MRA techniques are under investigation. There have been no complications. We plan to continue to recruit patients to this protocol over the coming year.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000084-04

LBC: DRD

Title: Diagnostic Efficacy of Virtual Bronchoscopy

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ronald M. Summers, MD, PhD (DDR, CC)

Collaborators, NIH: Steven Finkelstein, MD (NCI)
David Schrump, MD (TOS, NCI)
Michael C. Sneller, MD (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 in 3D. 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 that will be 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 three dimensional surface rendering of the airways and transfer of the digital data to videotape. In one of 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 only have fiberoptic bronchoscopy for clinically indicated purposes. We anticipate that virtual bronchoscopy will be diagnostically efficacious for disorders which 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 CL000085-04

LBC: DRD

Title: Normal Volunteer Scanning on Magnetic Resonance

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Choyke, MD (DDR, CC)

Supervisor of Record: King C. Li, MD (DDR, CC)

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

Collaborator, NIH: Larry Yao, MD (CC)

Total Staff Years: .7

Human Research: Human subject research

Keywords: MRI, magnetic resonance imaging

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 include optimizing contrast administration rates during magnetic resonance angiograms, automatic table motion techniques for peripheral run-off magnetic resonance angiography (MRAs), 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 CL000087-03

LBC: DRD

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Choyke, MD (DDR, CC)

Supervisor of Record: King C. Li, MD (DDR, CC)

Collaborators, Lab: Bradley W. Dick (DDR, CC)
Vincent B. Ho, MD (DDR, CC)
Wayne Olan, MD (DDR, CC)
Yantian J. Zhang (DDR, CC)

Collaborator, NIH: Andrew E. Arai, MD (LCE, NHLBI)

Total Staff Years: .5

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 a 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.

LBC: DRD

Title: Assessment of Renal Artery Stenosis and Renovascular Hypertension by Contrast-enhanced Magnetic Resonance Imaging

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Peter Choyke, MD (DDR, CC)

Supervisor of Record: King C. Li, MD (DDR, CC)

Collaborators, Lab: George R. Altizer (DDR, CC)
Clara Chen (DDR, CC)
Vincent B. Ho, MD (DDR, CC)
Jeffrey B. Kopp, MD (DDR, CC)
Lalith Talagala, PhD (DDR, CC)
Christopher Wilcox (DDR, CC)

Total Staff Years: .7

Human Research: Human subject research

Keywords: Assessment of RAS, renovascular hypertension

Summary: The purpose of this protocol is to determine whether magnetic resonance angiography (MRA) and captopril magnetic resonance 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 16 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 CL008038-03

LBC: LDRR

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Frank, MD, MS (LDRR, CC)

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

Collaborators, NIH: Alan Charles McLaughlin, PhD (SCS, NIMH)
Frank Ye, MS (DIRP, NIMH)

Total Staff Years: 2

Human Research: Human subject research

Keywords: Functional magnetic resonance imaging, 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 6 percent CO_2 and used to calibrate experiments for determining oxygen consumption). 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 CL090001-10

LBC: LDRR

Title: Magnetic Resonance Imaging in Multiple Sclerosis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Frank, MD, MS (LDRR, CC)

Collaborators, Lab: Craig N. Bash, MD (LDRR, CC)
Thomas R. Howard (LDRR, CC)
Bobbi K. Lewis (LDRR, CC)
Nancy Richert, MD, PhD (LDRR, CC)

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

Total Staff Years: 3.85

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. Anti-Tac antibodies in combination with Interferon beta have resulted in improvement of clinical and MRI MS disease activity in patients who were previously considered non-responders to conventional therapy. 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. Stage 1of this two-part study has been completed in more advanced MS patients, and there was no change or increase in the patients' MRI disease activity. Enrollment has started of active early relapsing remitting MS patients into this study. Changes in enhancing lesions compared to baseline disease activity will be used as the primary outcome measure. Cerebral atrophy represents the summation of all ongoing macroscopic and microscopic pathologic processes, including inflammation, demyelination, and neuronal and axonal loss in patients with MS. MS patients have approximately a two- to threefold increase in the rate of progression of cerebral atrophy compared with age-matched healthy controls. Although high-resolution three-dimensional MRI techniques have been used to study cerebral atrophy in other central nervous diseases such as Alzheimer's, these techniques have not been applied to the study of MS patients. Using standard clinical MRI examination for evaluating MS patients, there was a significant difference in the rate of progression of atrophy depending on the MRI pulse sequence used or the image analysis algorithm used in 10 relapsing remitting MS patients. These results indicated that the rate of cerebral atrophy may progress at rates from 0.5 to 1.5 percent per year depending on which MR images were evaluated and that standards need to be adopted if changes in the rate of progression of cerebral atrophy are to be used as an outcome measure in clinical trials. In addition, we have reported a significant decrease in brain volume as a result of treatment with intravenous steroids for acute exacerbations in MS patients. Prospective longitudinal studies are need to determine how measures of cerebral atrophy can be used to monitor treatment effects in individual patients with MS.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090003-08

LBC: LDRR

Title: Functional and Metabolic Imaging in the Brain

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Frank, MD, MS (LDRR, CC)

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

Collaborators, NIH: Alan Charles McLaughlin, PhD (SCS, NIMH)
Frank Ye, MS (DIRP, NIMH)

Total Staff Years: 1.3

Human Research: Human subject research

Keywords: Imaging of the brain, magnetic resonance imaging

Summary: Functional and metabolic magnetic resonance imaging (MRI) techniques have been rapidly evolving and have tremendous potential for research on clinical brain disorders. Clinical activation fMRI studies are performed at 1.5 and at 3.0 Tesla using blood oxygenation level dependent (BOLD) contrast method and arterial spin tagging (AST) techniques. Reproducible alterations in cerebral blood flow (CBF) were observed in healthy controls receiving intravenous infusions of a cyclo-oxygenase inhibitor (COX) 1, indomethicin, with almost complete suppression of the alteration of CBF to 6 percent carbon dioxide at rest. In contrast, high-dose oral COX 2 inhibitors did not suppress CBF measures in healthy controls, which may be due to either an inability to interact with receptors in the brain or possibly to an insufficient dose or poor absorption of the medication. We are planning to perform these studies during sensorimotor task activation in order to determine whether a COX 1 inhibitor suppresses the CBF response with stimulation. Future work will focus on improving the AST pulse sequences with back ground suppression to provide coverage over the whole head and also move the techniques to higher field strengths.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090004-09

LBC:	LDRR
Title:	Development and Evaluation of Magnetic Resonance Contrast Agents
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Joseph A. Frank, MD, MS (LDRR, CC)
Collaborators, Lab:	Syed Arbab Ali (LDRR, CC) Stasia A. Anderson (LDRR, CC) Lindsey Allison Bashaw (LDRR, CC) Jeff W.M. Bulte, PhD (LDRR, CC) E. Kay Jordan, DVM (LDRR, CC) Bobbi K. Lewis (LDRR, CC) Holly Zwickie, BS (LDRR, CC) Peter van Gelderen, PhD (LDRR, CC)
Collaborators, NIH:	Martin W. Brechbiel, PhD (ROB, NCI) L. Henry Bryant, PhD (CC)
Collaborator, Extramural:	Trevor Douglas, PhD (Chemistry, Temple University)
Total Staff Years:	4.5
Human Research:	Neither human cells nor tissues
Keywords:	Magnetic resonance contrast agents

Summary: STAR BURST Dendrimers (D), and superparamagnetic iron oxide nanoparticles (SPIO) are being developed as magnetic labels for *in vivo* cellular imaging. By combining commonly used transfection agents (TA) that have high net electrostatic charges with macromolecular high generation (G) dendrimers (G = 5, 7, 9, 10) were conjugated to DOTA and Gadolinium (III) ion or SPIO, effectively alters the ability of water (by shielding water molecules) from interacting with the contrast agents, nuclear magnetic resonance relaxation properties were detected. The physical chemical properties of the TA-SPIO molecules have been modeled and characterized and used to predict the electrostatic interaction between TA and the contrast agents and therefore the combination as a novel method for chaperoning contrast agents into endosomes in cells. Biodistribution studies of magnetically labeled human mesenchymal stem cells in rats demonstrated that labeled cells could be detected in the liver using a 1.5 Tesla clinical magnetic resonance (MR) unit for up to 14 days following an intravenous infusion of 900,000 cells. Biochemical and molecular biological analysis of TA-SPIO labeled stem cells indicates that cell labeling did not affect proliferation or viability of the stem cells nor the cells' ability to terminally differentiate under appropriate conditions. Using a similar magnetic labeling approach, enchephalotigenic T cells were injected into recipient mice and experimental allergic encephalomyelitis (EAE) was induced in these animals. Labeled T cells could be detected in the spinal cords of EAE mice at time of initial neurological event using MR microscopy at 7 Tesla. Immunohistochemical analysis revealed that TA-SPIO labeled T cells had similar proliferation assays and cytokine profiles as unlabeled encephalotigenic T cells. In addition, there was excellent correlation between MR microscopy and histology of spinal cords in clinically affected animals. Further studies are planned to determine if the trafficking of magnetically labeled stem cells or genetically altered cells can be detected by magnetic resonance imaging and whether these cells will localize to, repair, repopulate, or treat central nervous system diseases.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090005-07

LBC: LDRR

Title: Magnetic Resonance Imaging in Experimental Allergic Encephalomyelitis and Remyelination

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Joseph A. Frank, MD, MS (LDRR, CC)

Collaborators, Lab: Syed Arbab Ali (LDRR, CC)
Stasia A. Anderson (LDRR, CC)
Lindsey Allison Bashaw (LDRR, CC)
Jeff W.M. Bulte, PhD (LDRR, CC)
E. Kay Jordan, DVM (LDRR, CC)
Heather R. Kalish (LDRR, CC)
Bobbi K. Lewis (LDRR, CC)
Holly Zwickie, BS (LDRR, CC)

Collaborator, NIH: Richard C. Saunders, PhD (LN, NIMH)

Total Staff Years: 3.9

Human Research: Neither human cells nor tissues

Keywords: Magnetic resonance imaging, allergic encephalomyelitis, remyelination

Summary: Magnetic resonance imaging (MRI) scans were performed in myelin-deficient animals in which magnetically labeled rat progenitor oligodendrocytes were implanted into lateral ventricles of the brain. MRI at clinically relevant magnetic field strengths was performed *in vivo* on brains and showed extensive migration of magnetically labeled grafted cells along the ventricles and into the olfactory lobe of these animals. Similar migration patterns were observed in the monophasic experimental allergic encephalomyelitis (EAE) rat model where iron oxide-labeled embryonic stem cells were injected into the lateral ventricles and differentiated labeled neural cells were found in the surrounding parenchyma. MR images were correlated with histopathologic staining for iron, myelin, oligodendrocytes, astrocytes, and microglia. Both the Prussian blue and myelin staining closely matched the area of contrast enhancement seen on the MR images. Magnetically labeled encephalotigenic lymphocytes were intravenously infused as part of an adoptive transfer model of EAE in the mouse. Infiltration of labeled cells into the spinal cord and nerve roots of neurologically impaired mice was detected using *in vivo* magnetic resonance microscopy at 7 Tesla. This is the first demonstration of tracking activated lymphocytes into the central nervous system in an autoimmune disease model and opens the possibility of monitoring the trafficking of pharmaceutically or genetically engineered cells into the brain to further the understanding of the pathophysiology of EAE and the preclinical evaluation of new cell-based therapies. Serial MRI studies performed in the marmoset model EAE are used for preclinical evaluation of heparin for multiple sclerosis (MS). Recently it has been recognized that there is an ischemic component associated with acute inflammatory EAE and MS lesions, with fibrin deposition along activated endothelial cells. Subcutaneous heparin is used to prevent fibrin deposition within vessels as well as being an anti-inflammatory agent and having direct effects on T cell and macrophage migration.

Preliminary results comparing low- and high-dose heparin to placebo in the EAE marmoset model using clinical, pathologic, and MRI evaluations as outcome measures revealed no therapeutic advantage in the heparin-treated versus placebo-treated animals. There was no difference in the between the high-dose and placebo arms of the study in the incidence of hemorrhage into the spinal cords. Future studies are planned using the EAE marmoset model as a basis for harvesting stem cells and transplantation to determine if these cells will stimulate remyelination in EAE lesions.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL090006-10

LBC: LDRR

Title: Multimodality Radiological Image Processing System

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ronald L. Levin, DSc (DDR, CC)

Supervisor of Record: King C. Li, MD (DDR, CC)

Total Staff Years: 1

Human Research: Neither human cells nor tissues

Keywords: Multimodality radiological image processing system, radiological image

Summary: During this year, all data from the MRIPS Archive and Retrieval System (MARS) have been migrated to the Clinical Center's new Picture Archiving and Communication System (PACS). The remaining MRIPS file, Web, and ftp servers have been upgraded. 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 10/01/2001 to 09/30/2002

Principal Investigator: Michael Green (DNM, CC)

Collaborator, Lab: Jurgen Seidel, PhD (DNM, CC)

Collaborators, NIH: Calvin A. Johnson, PhD (ISL, CIT)
James V. Sullivan (MIF, BEIP, OD)

Collaborator, Extramural: Fernando Barbosa, EE
(Thomas Jefferson National Laboratory)

Total Staff Years: 1.8

Human Research: Neither human cells nor tissues

Keywords: Small animal PET, small animal SPECT, small animal radionuclide imaging

Summary: A second ATLAS II small animal positron emission tomography (PET) scanner was completed during this reporting period. The first and second ATLAS 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 to be easily and inexpensively upgraded to much higher spatial resolution by virtue of the much improved spatial linearity of the C-12 tube. ATLAS I, completed during the preceding reporting period, is now in use in the intramural research program to study organ function in small animals such as rats and genetically altered mice. Expanding collaborative studies between the Imaging Physics Laboratory and intramural investigators now include multi-modality imaging (PET, computed tomography [CT], OMRI and electron paramagnetic resonance) experiments in tumor-bearing mice, FDG and receptor imaging studies in the mouse and rat brain, and studies of new positron-labeled pharmaceuticals created by the PET Department. During this reporting period, work continued on enhancements and improvements to the ATLAS data acquisition software, user interface, and applications program set, including addition of new data acquisition protocols, a remote three-dimensional OSEM supercomputer image reconstruction option, and an extended image analysis and visualization package with multi-modality image registration capability. This latter addition is now being used to view spatially registered PET and CT scans of the same animal. These CT scans are obtained with a new small animal CT volume scanner installed during this reporting period immediately behind, and coaxial with, the ATLAS gantry. Registered CT/PET images will be used to correct the PET scans for radiation attenuation and to help identify structures labeled with the PET radiopharmaceutical.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000416-04

LBC: NMIP

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Stephen L. Bacharach, PhD (IPS, CC)

Collaborators, Lab: Joann M. Carson (IPS, CC)
Senthil Kumar, MD (IPS, CC)

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

Total Staff Years: 1.1

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 (NCI) and the Department of Radiology, performs clinical research in the use of imaging in oncology and in several other disease processes. In particular, NCI is studying the use of positron emission tomographic (PET) images, in conjunction with computed tomography (CT) and magnetic resonance (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 both determination of tumor morphology and the optimal determination of functional parameters such as blood flow, metabolism, and blood volume. The overall goal is the development of a clinically useful methodology 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. (1) Assessment of the physiologic models employed for blood flow measurement, using O-15 water. Several models are being analyzed, especially in regard to their utility in producing functional flow images. In addition, the results of these PET flow models are being compared to 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. Current work is focused on better models to account for tumor heterogeneity. (2) Methods for making accurate, quantitative measurements of FDG metabolism. Several schemes are being explored to compare the simple SUV (standardized

uptake value) method with Patlak analysis and to explore methods to simplify the kinetic model method while retaining accuracy. Initial results of this work have been recently accepted for publication in the *European Journal of Nuclear Medicine* and in the *Journal of Nuclear Medicine*. In addition, a method for making parametric images of glucose metabolism in tumors has been developed. Initial results were described in an oral presentation at the Society of Nuclear Medicine. (3) The above methods, combined with partial volume corrections from CT and factor analysis/principal components analysis, will be employed to make objective assessments of the various physiologic parameters (e.g., FDG uptake), and ROS analysis used to determine which of these quantitative indices it best for detecting disease, and to determine if such quantitative measures are better than subjective visual assessment. These studies will be performed in conjunction with Dr. I. Buvat at INSERM in Paris.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL000600-09

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, MD

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

Collaborators, NIH: Martin Brechbiel, PhD (ROB, NCI)
Ira Pastan, MD, PhD (LMB, NCI)
Thomas A. Waldmann, MD, PhD (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 (NCI) 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 used humanized anti-tac monoclonal antibody, is ongoing. A collaborative radioimmunotherapy trial with bone marrow support is ongoing with NCI (Drs. Bishop and Pastan). Various protocols using [F-18] FDG in positron emission tomograhy (PET) and [O-15] water for tumor detection, follow-up, and blood flow measurements are ongoing with NCI surgery Branch (Dr. Libutti). There are four ongoing collaborative studies evaluating fluorodeoxyglucose-PET for assessing tumor response to treatment (Dr. Swain, Dr. Mackall, Dr. Sausville, and Dr. Wilson). We have evaluated the use of fluorodeoxyglucose-PET in assessing sites of viral replication in patients with HIV (Dr. Brust). We have utilized FDG-PET to evaluate sites of metabolic activity in patients with systemic lupus erythematosus. We have performed preclinical studies evaluating pretargeting of antibodies for tumor therapy and have demonstrated therapeutic responses with Y-90 and Bi-213 in animal tumor models. Two antibody-strepavidin conjugates have been evaluated preclinically and papers have been accepted for publication. We are evaluating a third anti-mesothelin-strepavidin conjugated antibody.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL025001-01

LBC: NMRR

Title: Optimization of Parameters to Improve Tumor-targeting Properties of Radiobiologicals

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Chang Hum Paik (DNM, CC)

Supervisor of Record: Jorge A. Carrasquillo, MD (DNM, CC)

Collaborators, Lab: Eui Sik Han (DNM, CC)
Hyung Sik Kim (DNM, CC)
Luke S. Park (DNM, CC)
Noriko Sato, PhD (DNM, CC)
Karen J. Wong (DNM, CC)

Collaborators, NIH: Ira Pastan, MD, PhD (LMB, NCI)
Thomas A. Waldmann, MD, PhD (MB, NCI)

Total Staff Years: 3.25

Human Research: Neither human cells nor tissues

Keywords: Radiobiologicals, tumor targeting, dendrimer-based radiopharmaceuticals. Syntheses and *in vivo* tests of radiobiologicals in rodents for tumor-targeting

Summary: The overall purpose was to improve the tumor-targeting properties of radiolabeled biologicals by optimizing chemical parameters. This year's research was centered on two projects. The first project was to modify a spherical polymer, generation 3 PAMAM dendrimer (G3, MW 6909) and use it as a universal carrier of radiolabels and biological molecules. Using 3-[I-125] iodobenzoate and norbiotinamidosuccinate as model molecules, we have optimized the conjugation level of these molecules to G3 and improved the whole-body clearance kinetics in mice by blocking the non-specific uptake of G3 to liver and kidney upon neutralization of the highly positive-charged G3 by succinylation. We further improved the clearance kinetics of the radiolabel by inserting a metabolizable linker, triglycine, between the label and G3 to a level that can be useful for a therapy approach. This approach can be applied to synthesize At-211 labeled G3-biotin for an α emitter therapy of cancer that is pretargeted with antibody-streptavidin. The second project was to label Annexin V (AV) with I-125, In-111, Y-86, and Tc-99m for the detection of apoptosis to monitor the progress of cancer therapy. Among these, Tc-99m and Y-86 labeled AV were of particular interest because of their ideal imaging properties.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060001-07

LBC: NMRR

Title: Gene-specific Radiotherapy

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Ronald D. Neumann, MD (DNM, CC)

Collaborators, Lab: Igor Panyutin, PhD (DNM, CC)
Thomas A. Winters, PhD (DNM, CC)
Irina Panyutin, MD (DNM, CC)

Collaborator, NIH: Victor Zhurkin, PhD (MSS, NCI)

Collaborators, Extramural: Mirynal Dizdaroglu, PhD (NIST)
Peter Jacob, PhD (GSF)
Petra Pfeiffer, PhD (University of Essen)

Total Staff Years: 3.1

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 as delivery vehicles. The principal innovation in our approach is that it is the specific DNA sequence of a gene within the genome of a cell that becomes the target of radiotherapy, not the total DNA of that cell. Gene-specific radiotherapy optimally utilizes the sub-nanometer effect range of Auger emitters to allow targeting of most of the radiodamage to a selected gene sequence while producing minimal damage to the rest of the genome and other cell components. This approach requires a carrier molecule that exhibits enough specificity for a selected DNA sequence to deliver the radionuclide to that specific sequence and not to other sites in the genome. As our initial carrier molecule we selected short synthetic oligonucleotides that are able to form a sequence-specific triple helix with the target sequence, so-called triplex-forming oligonucleotides (TFO). This year we focused on the improvement of intracellular delivery of TFO via conjugation with nuclear localization signal (NLS) peptide. As an important step in the progression of gene-specific radiotherapy, we have demonstrated the ability of 125I-TFO-NLS conjugates to produce double strand breaks in a specific site in the human multidrug resistance (mdr1) gene within live cultured cells. We also studied the distribution of DNA strand breaks produced by decay of 125I and the repair of these breaks by protein extracts from mammalian cells. We found that the repair of the radiodecay-produced breaks was orders of magnitude less effective than that of the breaks produced by restriction enzymes and was always associated with deletions at the target site. The above findings prove the principle of gene-specific radiotherapy. To further improve the efficiency of our approach, we are currently developing a new class of delivery molecules based on peptide nucleic acids (PNA). In addition, we are developing a new mutation-based cell culture system for fast evaluation of Auger emitter carrying molecules. We have also completed development and

characterization of a proposed *in vitro* DSB repair assay employing DNA substrates bearing authentic DSB damage. The assay has been evaluated for optimal biochemical conditions and tested with a variety of cellular extraction techniques and human DSB repair enzyme preparation methods. Nonhomologous end joining (NHEJ), the primary human DSB repair pathway, has been shown to be responsible for DSB repair observed in our assay, and the assay has been used to demonstrate tumor progression dependent changes in NHEJ activity with human breast cell lines. These results suggest a potential role for this assay in individualization of cancer therapies by directly testing the DSB repair capacity of patient tumors. We have also employed our *in vitro* DSB repair assay to establish that the structure of the DSB produced by different DNA damaging agents (enzymatic, chemical, low-LET radiation, and 125-I) directly affects the ability of human enzymes to repair breaks. These findings are significant because the biological effects of radiation are thought to be a direct effect of the chemical structure of the DSBs produced by radiation, in conjunction with the inherent DSB repair capacity of the cells in which the breaks occur. Consequently, detailed knowledge of the chemical structure of a radiation-induced DSB would not only permit analysis of the biochemical mechanisms involved in its repair, but may permit application of such structural information to the direct manipulation of the cellular mechanism (DSB repair) responsible for resistance to many antineoplastic agents. Thus we have begun a study to map and define the complete spectrum and distribution of DNA lesions associated with 125-I-TFO-induced DSBs. Initial work from this study indicates that 125-I-TFO-induced DSBs are associated with base damage and other DNA lesions proximal to the DSB ends. Using our *in vitro* DSB repair assay, we have shown such structures to be strong inhibitors of human NHEJ repair. Completion of the 125-I DSB structural model will open many new avenues of investigation, including DSB structural effects on NHEJ, intracellular signaling cascades, apoptosis, and cellular sensitivity to DNA-damaging agents. They may also allow molecular analysis of repair processing at highly complex DSB structures. Such studies are not currently possible due to a lack of knowledge concerning the actual structure of a complex radiation-induced DSB and what aspects of its structure are biologically important.

POSITRON EMISSION TOMOGRAPHY DEPARTMENT

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LBC: NPET

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: William C. Eckelman, PhD (PETD, CC)

Collaborators, Lab: Richard E. Carson, PhD (PETD, CC)
Peter Herscovitch, MD (PETD, CC)
Elaine Jagoda, MS (PETD, CC)
Dale Kiesewetter (PETD, CC)
Lixin Lang (PETD, CC)
Ying Ma, PhD (PETD, CC)
Lawrence P. Szajek (PETD, CC)

Total Staff Years: 4.7

Human Research: Human subject research

Keywords: Radiopharmaceuticals, positron emission tomography

Summary: The Positron Emission Tomography Department has now performed the seminal experiment to prove that the radioligand F-18 FP-TZTP selectively binds to the M2 subtype muscarinic cholinergic receptor. M2 muscarinic receptors in frontal cortex and hippocampus are reported to decrease in Alzheimer's disease. Availability of selective M2 receptor ligands with positron-emitting labels would enable PET studies to assess the progression of the disease. ([F-18]FP-TZTP) is a muscarinic agonist with reported M2 selectivity. A muscarinic receptor radioligand, 3-(3-(3-fluoropropyl)thio)-1,2,5,thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (FP-TZTP) radiolabeled with the positron emitting radionuclide F-18 ([F-18] FP-TZTP) displayed regional brain distribution consistent with M2 receptor densities in rat brain. The purpose of the present study is to further elucidate the subtype selectivity of [F-18]FP-TZTP using genetically engineered mice that lack functional M1, M2, M3, or M4 muscarinic receptors. Using ex vivo autoradiography, the regional brain localization of [F-18] FP-TZTP in M2 knockout (M2 KO) was significantly decreased (51.3 percent to 61.4 percent; $P < 0.01$) when compared to the wild-type (WT) mice in amygdala, brain stem, caudate putamen, cerebellum, cortex, hippocampus, hypothalamus, superior colliculus, and thalamus. In similar studies with M1 KO, M3 KO, and M4 KO compared to their WT mice, [F-18]FP-TZTP uptakes in the same brain regions were not significantly decreased at $P < 0.01$. However, in amygdala and hippocampus, small decreases of 19.5 percent and 22.7 percent, respectively, were observed for M1 KO vs. WT mice at $P < 0.05$. Given the fact that large decreases in [F-18]FP-TZTP brain uptakes were seen only in M2 KO vs. WT mice, our data indicate that [F-18]FP-TZTP selectively labels M2 receptors *in vivo*. This radiopharmaceutical is currently in clinical trails in collaboration with National Institute of Mental Health scientists. Further clarification of the competitive blocking studies using non-radioactive [F-18]FP-TZTP was accomplished. Muscarinic agonists could alter CBF but in PET studies, [F-18]FP-TZTP is administered in tracer doses. Its specificity, however,

was tested by competitive binding inhibition with loading doses of P-TZTP, the non-fluorinated analog of the labeled ligand and an established M2-selective agonist. Effects of loading doses of P-TZTP on CBF were, however, not yet determined. We therefore measured CBF with the [C-14] iodoantipyrine method and laser-Doppler flowmetry in rats following administration of either 50 or 500 nmols/rat of P-TZTP, doses used in the saturability studies. Arterial blood pressure (MABP) fell markedly immediately after P-TZTP administration but recovered within 1 minute, and cortical CBF fell and rose synchronously with MABP. Local CBF decreased significantly immediately after the P-TZTP injection in two cerebral structures but returned to normal by 30 min after administration of either dose. A decrease in CBF may enhance the effects of blocking doses of P-TZTP in saturability studies, thereby overestimating the percentage of specific binding, although this effect will be small. The PET Department has also developed a radiolabeled nucleoside that can be used as either a proliferation agent or a reporter probe. An ester form of the radiotracer has been developed to monitor proliferation in the brain in the presence of a normal blood brain barrier. [Br-76]FBAU is a potential PET tracer for assessing proliferation. This study proposes that [Br-76]FBAU 3',5'-dibenzoate has higher blood-brain-barrier permeability than [Br-76]FBAU itself and thus might be better suited for application in the brain. [Br-76]FBAU 3',5'-dibenzoate was relatively stable in the plasma, gradually being hydrolyzed to [Br-76]FBAU. Biodistribution in rat showed that [Br-76]FBAU 3',5'-dibenzoate had higher brain uptake (0.119 ± 0.023 DUR at 1 h, versus 0.061 ± 0.006 for [Br-76]FBAU, $p = 0.003$, $N = 5$). The brain uptake indexes measured after carotid injection (29.6 ± 13.9 for [Br-76]FBAU 3',5'-dibenzoate, versus 10.0 ± 8.7 for [76Br]FBAU, $p = 0.012$, $N = 7$) support this claim. The DNA incorporation of [Br-76]FBAU was also confirmed. The results presented support the hypothesis that the dibenzoyl esters will result in higher brain uptake. The PET Department has also studied radiotracers in the new small animal imaging devices developed in the Department of Nuclear Medicine. *In vivo* imaging using PET is important in the development of new radiopharmaceuticals in rodent animal models for use as biochemical probes, diagnostic agents, or in drug development. If small animal imaging studies in rodents are to have the same quality as human PET studies, the same number of coincidence events must be detected from a typical rodent imaging voxel as from the human imaging voxel. To achieve this, roughly the same total amount of radiopharmaceutical must be given to the animal as to a human subject. At high specific activities, the mass associated with the human doses may not decrease the uptake of radioactivity at non-saturable sites or sites where an enzyme has a high capacity for a substrate. However, in the case of binding sites of low density such as receptors, the increased mass injected could saturate the receptor and lead to physiologic effects and non-linear kinetics. Because of the importance of the mass injected for small animal PET imaging, we experimentally compared (by biodistribution and phosphorimaging) high and low mass preparations of three compounds: 2-fluoro-2-deoxyglucose (FDG), 6-fluoro-L-metatyrosine (FMT), and a receptor-directed compound, the serotonin 5HT1A receptor ligand trans 4-fluoro-N-{2-[4-(2-methoxylphenyl) piperazino] ethyl}-N-(2-pyridyl) cyclohexanecarboxamide (FCWAY). Changes in the mass injected per rat did not affect the distribution of FDG, FMT, or, in the range of 0.6 nmol to 1.9 nmol, FCWAY. Large changes in the target to nontarget ratio were calculated for injected masses of FCWAY in the range of ~5 nmol per rat. If the specific activity of such compounds and/or the sensitivity of small animal scanners are not increased relative to human studies, small animal PET imaging will not correctly portray the “true” tracer distribution. These difficulties will only be exacerbated in animals smaller than the rat (e.g., mice). The PET Department has employed liquid chromatography/mass spectrometry to develop new analytical techniques that define the chemistry of

metabolites formed *in vitro* and *in vivo.* The PET Department has previously reported the development of fluorine-18 radiolabeled FCWAY (trans 4-FCWAY [(N-(2-(1-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl)-N-(2 pyridyl) trans 4-fluorocyclohexanecarboxamide]) as a high-affinity ligand for imaging the 5HT-1A receptor *in vivo*. We have developed three new analogues of FCWAY in a search for radiopharmaceuticals with unique imaging applications using PET. Two of the analogues were generated by replacing the fluorocyclohexane carboxylic acid with fluorobenzoic acid (FBWAY) or with 3-methyl-4-fluorobenzoic acid (MeFBWAY). The final analogue was generated by replacing the pyridyl group with a pyrimidyl group and the fluorocyclohexane carboxylate with fluorobenzoic acid (FPWAY). We evaluated the metabolic profile of these compounds using either human or rat hepatocytes to produce metabolites and LC/MS/MSto identify these metabolites. These *in vitro* metabolism studies indicate that hydrolysis of the amide linkage is the major metabolic pathway for FPWAY and FBWAY in human hepatocytes, whereas aromatic ringoxidation is the major metabolic pathway for MeFBWAY. Aromatic ring-oxidation is the major metabolic pathway for all three analogs in rat hepatocytes. The value of these *in vitro* metabolic studies is the ability to demonstrate species differences prior to the acquisition and interpretation of *in vivo* results.

LABORATORY MEDICINE DEPARTMENT

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LBC: DLM

Title: Analytical Methodology: Development and Interpretive Application

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Nadja Rehak (CCS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

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

Total Staff Years: .1

Human Research: Neither human cells nor tissues

Keywords: Analytical methodology

Summary: Handheld glucose meters are intended for monitoring patients with diabetes mellitus at home or other extra-laboratory settings. The recommended method for most meters is to apply a small drop of fresh capillary whole blood directly to the glucose test strip. The measurement is based on glucose oxidase reaction that consumes a stoichiometric amount of molecular oxygen. Because of their ease of use and rapid turnaround time, many hospitals use glucose meters for point-of-care testing (POCT) of critically ill patients. However, very low and high hematocrit and oxygen tension that may be present in these patients' samples have been reported to cause inaccurate results. We investigated the effects of different hematocrit and oxygen tensions on glucose measurements with the latest generation of test strips (Lifescan SureStepPro, Johnson & Johnson Company) that are used for POCT of critically ill patients in the Clinical Center. Heparinized venous whole blood was prepared to contain hematocrit between 10 and 68 percent (reference range: 32 to 48 percent), glucose concentrations between 22 and 495 mg/dL, and oxygen tension between 30 and 245 mm Hg. The samples were analyzed simultaneously with SureStepPro test strip (glucose) and with ABL 725 Clinical System (oxygen tension and glucose). The test strip glucose results, expressed as the difference (percent) from the ABL results, were not affected by the oxygen tension in the sample ($p > 0.2$). The agreement between the test strip and ABL results was acceptable for samples with normal hematocrit (slope = 1.04, intercept =1.3). However, at hematocrit <32 percent the test strip overestimated the glucose concentration (slope=1.18, intercept=2.6). For samples with glucose concentration above 100 mg/dL and hematocrit <20 percent the overestimation was statistically ($p< 0.007$ and clinically (up to 40 percent) significant. Based on these results the SureStepPro Test strip can be used to measure venous whole blood glucose but should not be used for patients with extremely low hematocrit.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010010-27

LBC: DLM

Title: Magnesium Metabolism in Humans and Biological Systems

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Nadja Rehak (CCS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

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

Collaborators, NIH: Charles Bolan, MD (DTM, CC)
Susan Leitman, MD (DTM, CC)

Total Staff Years: .4

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 magnesium (Mg2+) effect 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 PTH, citrate, ionized Mg (iMg) and ionized Ca (iCa), and other electrolytes that occur during leukapheresis. Laboratory analyses of serum and urine specimens were performed during allogeneic peripheral blood progenitor cell donations (n = 244) with and without prophylactic Ca. Marked increases in serum citrate concentration occurred that were negatively correlated with significant decreases in iCa and iMg (by up to 56 percent). The renal excretion of citrate was accompanied by significantly increased renal excretion of Ca and Mg. The serum concentration of both Ca and Mg remained decreased 24 hours after the procedure. Symptoms of citrate toxicity were more frequent in donors who had low serum total Mg concentration at the start of procedure. Prophylactic Ca infusions reduced the symptoms and attenuated the decreases in serum potassium.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010220-10

LBC: DLM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: McDonald K. Horne, III (HEME, CC)

Collaborators, 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 more recently we have been using the protein in a variety of studies. 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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Margaret E. Rick, MD (HEME, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

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

Total Staff Years: .1

Human Research: Human subject research

Keywords: von Willebrand 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. Specific regions of the patient's vWf gene were amplified by polymerase chain reaction (PCR), 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 then used a PCR mutagenesis technique to insert the mutation into cloned DNA and expressed the abnormal protein. The latter was tested in an assay for binding factor VIII and was shown to manifest decreased binding. A manuscript containing the expression data is in preparation. Two unrelated patients with von Willebrand 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 CL010247-08

LBC: DLM

Title: Molecular and Phenotypic Methods for Identifying Mycobacteria and Nocardia

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Frank G. Witebsky, MD (MS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, Lab: Patricia S. Conville, MS (MS, CC)

Collaborators, NIH: Steven M. Holland, MD (LHD, NIAID)
Victoria L. Anderson (LHD, NIAID)

Collaborators, Extramural: June M. Brown, BS (CDC)
Karen C. Carroll, MD (Department of Pathology, The Johns Hopkins Hospital)
Joann Cloud, MS (ARUP Institute)
Arnold G. Steigerwalt, BS (CDC)

Total Staff Years: .8

Human Research: Neither human cells nor tissues

Keywords: Polymerase chain reaction, restriction fragment length polymorphism, DNA-DNA hybridization, mycobacteria, norcardia

Summary: Polymerase chain reaction (PCR) amplification of a portion of the genome of both rapidly growing mycobacteria and nocardiae, followed by restriction fragment length polymorphism (RFLP) analysis of the amplification products, has proven to be a useful technique in the diagnostic laboratory. These organisms can be identified to the species level within a few days of organism isolation, 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 several clinical isolates belonging to species of Nocardia that have not, so far as we know, been previously reported to cause disease in patients in the western hemisphere. A manuscript describing the RFLP component of our methodology has been published. DNA-DNA hybridization is currently being used to define more precisely which of our isolates belong to unusual but already-described species, and which might belong to hitherto undescribed species. A characterization of these isolates based on phenotypic and other molecular biologic features is also under way. The fact that some unusual Nocardia species can be pathogenic in patients with chronic granulomatous disease has also been noted in another manuscript.

LBC: DLM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Steven H. Fischer, MD, PhD (DLM, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, NIH: John E. Bennett (LCI, NIAID)
Karoll Cortez (NIAID)
Gary A. Fahle (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 developed 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 improve precision and, therefore, potential predictive value for disease onset or progression, we have developed a real-time CMV PCR assay. This assay uses 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. We have conducted a prospective study using the real-time CMV PCR assay to test whole blood samples from bone marrow transplant patients. Preliminary analysis of the prospective study data reveals that the real-time PCR assay has a high degree of sensitivity for detecting viremic episodes that are detected by CMV antigen and a negative predictive value of greater than 95 percent for CMV antigen-negative specimens.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010269-07

LBC: DLM

Title: Markers of Disease Activity in Idiopathic Inflammatory Myopathy

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Margaret E. Rick, MD (HEME, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, Lab: Ann M. Cullinane (HEME, CC)

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

Collaborator, Extramural: Lisa Rider (FDA)

Total Staff Years: .03

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 include 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 patient shows an increase in thrombin-antithrombin complexes, which is a sensitive assay for activation of coagulation factors. This project has been awaiting input from investigators in the National Institute of Arthritis and Musculoskeletal and Skin Diseases. Further testing is being planned.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010279-06

LBC: DLM

Title: Comparison of Microbiologic and Cytologic Results for Bronchoalveolar Lavages

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Frank G. Witebsky, MD (MS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, Lab: Frida Stock, BS (MS, CC)

Collaborators, NIH: Elizabeth M. O'Shaughnessy, MD (NCI)
Thomas J. Walsh, MD (NCI)

Total Staff Years: .02

Human Research: Human cells or tissues

Keywords: Bronchoalveolar lavages

Summary: Bronchoalveolar lavage specimens are usually split between cytology and microbiology for laboratory analysis. Because these two laboratories use different methodologies in the work-up of these specimens, we thought it would be useful to review the results each laboratory obtained on these specimens. 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 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 mycobacteria and Pneumocystis carinii. Further analysis of the data had been temporarily postponed to deal with more pressing projects. The analysis of the data, including data from more recent years, has recently been resumed, and we hope to complete the project during the next year.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010281-06

LBC: DLM

Title: Detection and Identification of Mycobacteria in Clinical Specimens

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Steven H. Fischer, MD, PhD (DLM, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, Lab: Charles Huber, BA (DLM, CC)

Collaborator, NIH: Gary A. Fahle (MS, CC)

Collaborators, Extramural: Susan Dorman, MD (Center for TB Research, Johns Hopkins)
Mark Manak, PhD (BBI-Biotech Research Laboratories, Inc.)
Jang Rampal, PhD (Advanced Technology Center, Beckman Coulter Corp.)

Total Staff Years: .2

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 days to months. The development of a few highly specific molecular probes for testing cultures growing acid-fast bacilli has greatly reduced the time to identify 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. It would be useful to have amplification assay systems that 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 an assay 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 begun 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 DLM under a cooperative research and development agreement with BBI Biotech. Using PCT as a pretreatment before nucleic acid extraction, we were able to increase the average polymerase chain reaction signals from suspensions containing Mycobacterium gordonae and suspensions of Mycobacterium tuberculosis. A collaborative prospective study is being initiated with the Johns Hopkins Center for TB Research and BBI to evaluate the utility of different PCT conditions for respiratory specimen processing before Mycobacterium detection by culture or molecular testing.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010283-06

LBC: DLM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Margaret E. Rick, MD (HEME, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, Lab: Kristen Hansmann (HEME, CC)

Total Staff Years: .15

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. 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 follow-up of patients before and after treatment in a study with the National Heart, Lung and Blood Institute in the treatment setting of T cell-depleted auto-stem cell transplantation in patients with severe ITP. Sixteen patients have been studied. An oral presentation was given at the national meeting of the American Society of Hematology in December 2001, and a publication is in press.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010287-06

LBC: DLM

Title: Assessment of Lymphocytes in Patients with Autoimmune Lymphoproliferative Syndrome

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Thomas A. Fleisher, MD (IMMUNE, CC)

Collaborators, Lab: Margaret R. Brown, MA (IMMUNE, CC)
Amie Elizabeth Bryson (IMMUNE, CC)

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

Collaborator, Extramural: Jack Bleesing, MD (Pediatrics, Arkansas Childrens Hospital Research Institute)

Total Staff Years: .35

Human Research: Human 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. The observations in the B cells of ALPS patients are tied directly to an additional active protocol directed at the assessment of B220 expression on human lymphocytes. The relative deficiency in CD4/CD25 T cells that we have identified has resulted in the initiation of functional studies directed at this T cell subpopulation to assess whether the immunophenotypic findings represent a functional defect in immunoregulatory T cells that could explain the genotype phenotype disparity in families with ALPS type 1a.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010300-04

LBC:	DLM
Title:	Identification of Proteolytic Activity for von Willebrand Factor
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Margaret E. Rick, MD (HEME, CC)
Supervisor of Record:	Thomas A. Fleisher, MD (DLM, CC)
Collaborators, Lab:	David Aronson (HEME, CC) Dennis M. Krizek (HEME, CC)
Collaborator, NIH:	William G. Stetler-Stevenson, MD, PhD (LP, NCI)
Collaborators, Extramural:	Stephan Moll (University of North Carolina School of Medicine) Mark Taylor (University of North Carolina School of Medicine)
Total Staff Years:	1
Human Research:	Human subject research
Keywords:	Proteolytic activity, von Willebrand factor

Summary: Proteolysis of von Willebrand 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 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 have studied a group of 50 masked plasmapheresis samples in collaboration with hematology investigators from the University of North Carolina medical school, and a paper is in press presenting the results of this study. We have begun a collaboration with Dr. William Stetler-Stevenson (National Cancer Institute) to attempt to express the protein in mammalian cells.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010302-04

LBC: DLM

Title: Development and Diagnostic Use of Rapid Immunoassays

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Alan T. Remaley, MD, PhD (CCS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

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 completed the development of a new intact parathyroid hormone (PTH) assay that does not cross react with the 7-84 fragment of PTH. We have compared the old and new rapid PTH assay and have found that there is faster decay of PTH with the new assay following parathyroidectomy. We have also completed an analysis of the possible diagnostic utility of a rapid adrenocorticotropic hormone assay on jugular venous samples. The results showed that such an assay would be useful for preventing the need for petrosal venous sampling in about 50 percent of the cases of Cushing's disease. In the coming year, we plan to further develop a rapid gastrin assay for localizing gastrinomas.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010303-04

LBC: DLM

Title: Development of New Assays for Lipoprotein Testing

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Alan T. Remaley, MD, PhD (CCS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, Lab: Maureen L. Sampson (CCS, CC)

Total Staff Years: .5

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 several manual steps. 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 have fully automated the assay on a standard clinical chemistry analyzer, and the NIH has licensed the technology to an outside company for potential commercialization. In the coming year, we plan to make further refinements of the assay and evaluate any commercial tests developed by the licensee. We also plan to investigate the use of a new colorimetric lipase substrate for routine diagnostic lipase testing.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010304-04

LBC: DLM

Title: Mutation Analysis of Selected Lymphoid Immune Disorders

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Thomas A. Fleisher, MD (IMMUNE, CC)

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

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

Total Staff Years: .6

Human Research: Human cells or tissues

Keywords: Lymphoid-immune disorders

Summary: This project represents an extension of a long-standing 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 direct gene sequencing with fluorescent probes. These studies have identified a number of new mutations in both diseases, and these data have been published or submitted for publication. During the past year additional disorders have been added to the menu for mutation analysis, including the CYBB gene coding for gp91phox that is deficient in X-linked CGD, the NEMO gene that is deficient in ectodermal dysplasia with hyper IgM syndrome, and the CD40 ligand gene that is defective in X-linked hyper IgM syndrome. 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. The project has also provided teaching opportunities for fellows in training.

LBC: DLM

Title: Analytical Performance and Clinical Utility of Thyroid Function Tests

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Gyorgy Csako, MD (CCS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, Lab: Rene A. Costello, MT (CCS, CC)
Alan T. Remaley, MD, PhD (CCS, CC)

Collaborators, NIH: Lynnette K. Nieman, MD (DIR, NICHD)
Monica C. Skarulis, MD (DIR, NIDDK)
Frank R. Pucino, Jr., PharmD (CC)
Nicholas J. Sarlis, MD, PhD (CEB, NIDDK)
Robert A. Wesley, PhD (OD, NCI)

Total Staff Years: .3

Human Research: Human subject research

Keywords: Thyroid function tests, thyroid cancer, meta-analysis, hemostasis, atherothrombosis, lipoprotein(a)

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 U.S. population 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 continued to assess 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. Long-term thyroid hormone therapy aiming at the suppression of serum thyrotropin (TSH) has been traditionally used in the management of well-differentiated thyroid cancer. However, formal validation of the effects of thyroid hormone suppression therapy through randomized controlled trials is lacking. Additionally, the effect of TSH at low ambient concentrations on human thyroid tumorigenesis remains unclear. We identified from the literature 28 clinical trials that dealt with the use of thyroid hormone suppression therapy in patients with thyroid cancer between 1934 and 2001. Applying a Likert scale, 15 of the 17 “interpretable” studies showed either a “likely” or “questionable” beneficial effect of thyroid hormone suppression therapy on the outcome of thyroid cancer. We quantitatively evaluated the effect of thyroid hormone suppression therapy on the likelihood of major adverse clinical events (disease progression/recurrence and death) in a cumulative thyroid cancer cohort from 10 studies that qualified for meta-analysis. The studies represented a combined total of 4,174 patients, of whom 2,880 (69 percent) were reported as being on thyroid hormone suppression therapy. According to meta-analysis, the group of patients on thyroid hormone suppression therapy had a decreased risk of major adverse clinical events (relative risk 0.73, confidence interval 0.60-0.88, $p < 0.05$). Thus, despite the known adverse effects of long-term administration of thyroid hormones, suppression therapy with these

hormones is justified in patients with thyroid cancer. High-sensitivity TSH assays that are capable of detecting very low TSH levels (at least < 0.10 mu/L and, possibly, < 0.01 mu/L) are needed to assess the adequacy of thyroid hormone suppression therapy in these patients. In another collaborative project, we analyzed the results of a pilot trial that evaluated the effects of extreme changes in thyroid hormone availability on serum lipids, lipoproteins, apolipoproteins, and on hemostasis in thyroid cancer patients receiving thyroid hormone suppression therapy. Thyroid function tests, serum lipid parameters (with special respect to lipoprotein[a]), and various hemostatic parameters were assessed before, at, and after the time these patients were undergoing scanning. Better understanding of the relationship between thyroid hormones and the lipid and hemostatic system is important because hypothyroidism is known to be associated with elevated serum lipids and increased incidence of atherothrombotic events.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010306-04

LBC: DLM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Gyorgy Csako, MD (CCS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, Lab: Rene A. Costello, MT (CCS, CC)
Rosario M. Delgado, MT (CCS, CC)
Alan T. Remaley, MD, PhD (CCS, CC)

Collaborators, NIH: Richard O. Cannon, MD (CB, NHLBI)
Susan Leitman, MD (DTM, CC)
Frank R. Pucino, Jr., PharmD (CC)
Robert A. Wesley, PhD (OD, NCI)

Collaborators, Extramural: Stephen E. Epstein, MD (Cardiovascular Research Institute, Washington Hospital Center)
Jianhui Zhu, MD (Cardiovascular Research Institute, Washington Hospital Center)

Total Staff Years: .9

Human Research: Human subject research

Keywords: Atherosclerosis, thrombosis, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein, lipoprotein(a), infection, inflammation, C-reactive protein

Summary: Studying the analytical performance of various commercial methods (two different enzyme-linked immunosorbent assays and an immunoturbidimetric method) for the measurement of lipoprotein(a), we observed analytically and clinically important differences in their standardization. In addition, we found evidence for apolipoprotein(a) isoform-dependence in at least two of three methods, which casts further doubt on their clinical utility. In a collaborative study, we studied the possible effect of frequent blood donations on the development of atherosclerosis in healthy adults. Despite reductions in body iron stores by frequent blood donation, there was no evidence for a reduced risk of atherosclerosis as assessed by carotid artery intimal thickness and a variety of multiple laboratory markers of inflammation. In another collaborative clinical study, we observed that combining an HMG-CoA reductase inhibitor drug ("statin") with estrogen attenuates the increase in C-reactive protein during estrogen replacement therapy in postmenopausal women. This is of importance because elevated C-reactive protein levels may have inflammatory and thrombotic consequences that compromise any benefit to cardiovascular risk reduction by estrogens. In a third collaborative clinical study, we assessed the effect of hormone replacement therapy on carotid artery compliance in healthy postmenopausal women. We found that a 3-month hormone replacement therapy significantly improved the carotid artery compliance, as determined by magnetic resonance imaging and blood pressure measurements. This effect was

independent of changes in serum levels of lipids and markers of inflammation, including C-reactive protein. In a fourth collaborative clinical study, we studied the role of angiotensin II type 1 (AT1) receptor in the regulation of cellular adhesion molecules in atherosclerosis. We found that AT1 receptor antagonism induced by the administration of losartan (an AT1 receptor antagonist) selectively modulated the expression of L-selectin on leukocytes, while it did not affect other leukocyte and serum adhesion molecules and C-reactive protein. L-selectin is a leukocyte adhesion molecule that is rapidly shed after leukocyte activation, so it is often decreased in coronary artery disease. In another collaborative clinical study, we continued studying the relationship between antibodies to various heat-shock proteins and coronary artery disease. Results of this study are being evaluated.

LBC: DLM

Title: Development and Clinical Application of Molecular Diagnostic Tests

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Gyorgy Csako, MD (CCS, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, Lab: Rene A. Costello, MT (CCS, CC)
Rosario M. Delgado, MT (CCS, CC)

Collaborator, NIH: Trey Sunderland, MD (NIMH)

Total Staff Years: .6

Human Research: Human cells or tissues

Keywords: Polymerase chain reaction, RFLP, SSCP, DNA, Alzheimer's disease

Summary: An increasing number of genes have been linked to Alzheimer's disease (AD) over the past decade. Recently, two different polymorphisms of the alpha-2-macroglobulin gene, residing on chromosome 12, were suggested to be associated with sporadic AD. One of these polymorphisms is based on a pentanucleotide deletion; the other is based on a point mutation by changing the nucleotide A to G (and the amino acid isoleucine to valine in the corresponding protein). The product of this gene is alpha-2-macroglobulin, a pan-proteinase inhibitor occurring in many organs and tissues, including the brain. Several studies have suggested that alpha-2-macroglobulin may have a protective effect against the deposition of beta-amyloids and may stimulate their degradation. Beta-amyloids now are considered to be major pathogenic factors in the cascade of events that lead to brain destruction, with resultant memory loss and other manifestations of AD. A third recently identified gene polymorphism that has been linked to AD involves a C to T nucleotide mutation in the cathepsin D gene located on chromosome 11. This mutation results in an amino acid sequence change of the gene's protein product from alanine to valine. The T allele may be associated with increased protein expression (increased pro-cathepsin D secretion) and altered intracellular maturation. Cathepsin D is a major intracellular aspartyl protease present in the endosomal-lysosomal system and has been shown to be involved in the pathogenesis of AD. Despite a number of studies supporting an association of the three different gene mutations (possibly all related to protein processing) with AD, there is also contrary evidence in the literature, and therefore the relationships remain controversial. During the past few years, we developed methods that are more practicable (PCR-SSCPs for the two alpha-2-macroglobulin mutations and PCR-SSCP and LightCycler methods for the detection of the cathepsin D mutation) than conventional PCR-RFLPs for the study of these gene polymorphisms. Using these methods, we recently completed screening of approximately 400 subjects, including patients confirmed to have AD and their family members, for the three gene polymorphisms. We are now analyzing whether there is a relationship between these gene mutations and the risk of AD. In contrast to the alpha-2-macroglobulin and cathepsin D gene mutations, the apolipoprotein 4 allele is a well-established risk factor for AD. We recently validated and implemented for routine use a fully automated, real-time ultrafast PCR method based on the LightCycler instrument for more reliable and cost-effective determination of the alleles of the apolipoprotein gene.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010308-03

LBC: DLM

Title: Assessment of Memory B Cells in Immune Disorders

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Thomas A. Fleisher, MD (IMMUNE, CC)

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

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

Collaborator, Extramural: Jack Bleesing, MD (Pediatrics, Arkansas Childrens 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. We are evaluating normal subjects and patients with specific immune disorders, including autoimmune lymphoproliferative syndrome (ALPS) and CGD. In addition, the immunophenotypic data are being compared with single B cell Ig gene somatic hypermutation results generated in Dr. P. Lipsky's laboratory (National Institute of Arthritis and Musculoskeletal and Skin Diseases). These investigations have established that CD27 expression is altered in CGD, and this alteration appears to be 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, which may be related to some extent to protein cleavage from the cell surface based on increased levels of soluble CD27 found in the plasma of ALPS patients. Recent findings suggest that memory B cell levels are normal in CGD based on normal frequency of somatic hypermutation in B cells despite the marked decrease in CD27 expression on the B cells. This contrasts with a virtual absence of memory B cells using the same indicator system in ALPS patients' B cells. These studies suggest that the Fas pathway may be critical in the generation of memory B cells, while defective NADPH oxidase activity does not affect memory B cell development but does diminish CD27 expression. These studies also point out that CD27 is not a consistently reliable marker of memory B cells in humans.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010309-03

LBC: DLM

Title: Assessment of B220 Expression on Human Lymphocytes

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Thomas A. Fleisher, MD (IMMUNE, CC)

Collaborators, Lab: Margaret R. Brown, MA (IMMUNE, CC)
Amie Elizabeth Bryson (IMMUNE, CC)

Collaborator, Extramural: Jack Bleesing, MD (Pediatrics, Arkansas Childrens Hospital Research Institute)

Total Staff Years: .5

Human Research: Human subject research

Keywords: B220 expression, human lymphocytes

Summary: The 220 KD isoform of CD45 recognized by the monoclonal antibody B220 is differentially expressed on lymphocyte subsets. Among B cells it appears to distinguish a subset of memory B cells as well as being expressed on non-isotype switched B cells that do not have a memory phenotype. Within the ALPS population, B220 is expressed on the characteristic alpha-beta TcR double-negative T cell. In addition, B220 positivity appears to herald lymphocyte apoptosis following lymphocyte activation. B220 expression is also noted in T cell large granular lymphocyte (LGL) leukemia. The expression of B220 represents a glycosylation modification of the CD45 molecule. These findings in the context of the specific clinical observations associated with B220 expression suggest that the control of CD45 glycosylation has a significant role in lympohcyte homeostasis and trafficking. Studies are being undertaken to elucidate these issues.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010316-02

LBC: DLM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Daniel Fedorko, PhD (MICRO, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

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

Collaborator, Extramural: Charles P. Cartwright, PhD (Clinical Microbiology, Hennepin County Medical Center)

Total Staff Years: .5

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 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 originally selected five primer pairs specific for the two toxin genes of *C. difficile*. We have selected the two primer pairs that are the most efficient in amplifying toxin A and toxin B. The base sequences for these two genes are both very AT rich, and we discovered that this prohibits the development of a sensitive LightCycler assay. We have developed an enzyme-linked immunosorbent assay format for our amplicon detection and are now in the process of determining the sensitivity of our assay before using patient specimens.

LBC: DLM

Title: Interaction of Plasminogen with Human Platelets

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Mcdonald K. Horne, III (HEME, CC)

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. We have 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, with the goal of identifying 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 CL010318-02

LBC: DLM

Title: Development of a Cellular Immune Function Program

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, NIH: Kenneth Hirsch MD (CC)
Jennifer M. Puck, MD (IG, GMBB, NHGRI)
Gulbu Uzel, MD (LHD, NIAID)

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Evaluation of 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 has been developed. It is now operational and is providing critical information in the evaluation of patients with possible immune disorders and in following patients post-bone marrow transplantation. This has been supplemented with additional testing to evaluate the T-cell antigen receptor repertoire using TcR spectratyping with 26 pairs of polymerase chain reaction primers directed at the 24 major families of TcR beta chains. These studies are being used in assessing patients with congenital immune deficiency disorders and evaluating these patients post-cellular reconstitution. T-cell receptor excision circle assessment has also been developed to assess post-bone marrow transplant patients' thymic output and to be applied to immune deficient patients undergoing gene therapy in the future.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010319-02

LBC: DLM

Title: Platelet Function in Patients Treated with SSRI Versus Non-SSRI Antidepressants

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Margaret E. Rick, MD (HEME, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, 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 cells or tissues

Keywords: SSRI anti-depressants, platelet function

Summary: Selective serotonin reuptake inhibitors (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, can identify the changes in platelet function associated with SSRI use.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010321-02

LBC: DLM

Title: Macromolecular Substrates for Enzymes

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Glen Hortin (DLM, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, NIH: Bonnie S. Meilinger, MT (CCS, CC)

Total Staff Years: .4

Human Research: Human cells or tissues

Keywords: Thrombosis, coagulation factors, thrombin proteases, amylase

Summary: Measurements of enzyme activity are 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 were described in *Clinical Chemistry* 2001;47:215-222. Continuing studies have examined further models analyzing effects of substrate size on enzyme activity. These studies have examined effects of substrate size on antibody inhibition of enzyme activity (*J Clin Lab Anal* 2001;15:64-70) and on the activity of enzymes that have an active site located within a tubular structure that serves as a size-dependent filter, using proteasomes as a model (*J Prot Chem* 2002;21:333-337). We have been working on better physical characterization of the size of the new synthetic substrates using light-scattering techniques and development of substrates for other classes of proteases such as metalloproteases.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010322-02

LBC: DLM

Title: Homocysteine and Cysteine Interactions

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Glen Hortin (DLM, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, NIH: Gyorgy Csako, MD (CCS, CC)
Bonnie S. Meilinger, MT (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. 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. Goals of these studies are 1) to identify which measurements of plasma homocysteine, cysteine, or combinations of amino acids 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 the function of plasma proteins. A starting point for examining interactions with proteins is to study the binding and exchange of cysteine and homocysteine with different plasma proteins.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010323-01

LBC: DLM

Title: Coagulant and Fibrinolytic Parameters in Postmenopausal Women

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Mcdonald K. Horne, III (HEME, CC)

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

Collaborator, NIH: Donna Jo McCloskey, RN (CC)

Total Staff Years: .4

Human Research: Human subject research

Keywords: Coagulation, fibrinolysis, menopause

Summary: To design longitudinal studies, the within-subject variability of the measured parameters must be known. However, information is limited about the within-subject variability of parameters of coagulant and fibrinolytic activity in postmenopausal women. This shortage restricts the range of studies that can be designed to assess the impact of hormone replacement therapy (HRT) on coagulation and fibrinolysis in this population. Therefore, we measured a battery of relevant factors in 34 healthy postmenopausal women on four separate occasions over 3 months. We found that certain parameters (e.g., plasminogen activator inhibitor-1) varied greatly over time within individuals (e.g., coefficient of variation [CV] 55 percent), whereas others (e.g., thrombin-antithrombin complex concentrations) varied much less (e.g., CV = 11 percent). The information we accumulated will be used to determine how frequently different parameters must be measured to detect significant biological changes that are temporally related to an intervention, such as the administration of HRT.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010324-01

LBC: DLM

Title: Evaluation of Hypercoagulability in Patients with Major Depression

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: McDonald K. Horne, III (HEME, CC)

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

Collaborators, NIH: Giovanni Cizza, MD, PhD (CNE, NIMH)
Phillip Gold, MD (NIMH)

Total Staff Years: .4

Human Research: Human subject research

Keywords: Depression, hypercoagulability

Summary: Because major depression is associated with an increased incidence of thromboembolic disease, we are measuring laboratory parameters of coagulation and fibrinolysis in cohorts of depressed patients and control subjects. There are two study groups: (1) individuals under long-term observation are being tested in the morning and evening to assess possible perturbations in the natural diurnal variation of factor VIII and plasminogen activator inhibitor-1 (PAI-1); and (2) individuals being infused with insulin and glucose to assess their insulin resistance are being tested before and after the infusions with measurements of factor VIII, thrombin-antithrombin complexes, and PAI-1.

Results: The normal diurnal variation of factor VIII, but not PAI-1, may be blunted in depressed patients compared to controls. Insulin infusion increases factor VIII and PAI-1, but the elevations are greater in depressed patients than in controls.

Conclusions: The increased incidence of thromboembolic disease in patients with major depression may be mediated by underlying hypercoagulability.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010325-01

LBC: DLM

Title: Evaluation of a Commercial Polymerase Chain Reaction Assay for Diagnosis of *Clostridium difficile* Colitis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Daniel Fedorko, PhD (MICRO, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, Lab: Pattarachai Kiratisin, MD (MICRO, CC)
Nancy A. Nelson (MICRO, CC)

Collaborator, Extramural: Charles P. Cartwright, PhD (Clinical Microbiology, Hennepin County Medical Center)

Total Staff Years: .04

Human Research: Human cells or tissues

Keywords: *Clostridium difficile*

Summary: Until recently, the only commercially available tests for diagnosis of *Clostridium difficile*-associated diarrhea were assays for the detection of toxins A and B. Although not FDA approved, there is now a commercially available kit to perform nested polymerase chain reaction (PCR) for detection of toxin A and/or B genes in patient stool specimens. The only available performance data regarding this assay are in a published abstract from a recent national meeting. We purchased kits from the Korean manufacturer and have begun to evaluate the performance of the assay compared to the traditional *C. difficile* detection methods culture and an enzyme-linked immunosorbent assay for toxins A and B. So far, this commercial assay compares well with the traditional methods. We will continue our comparison until we have tested 300 specimens by all methods. We have developed our own PCR assay for the detection of *C. difficile* toxin A and B genes, and we plan on using this assay to resolve discrepant results. A chart review will be performed to determine the clinical relevance of results generated by the commercial diagnostic PCR kit. To date there are no published articles describing the performance of this PCR kit.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010326-01

LBC: DLM

Title: Identification of Streptococcus Mitis Species by Housekeeping Gene Sequencing

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Steven H. Fische, MD, PhD (DLM, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborator, Lab: Pattarachai Kiratisin, MD, PhD (DLM, CC)

Total Staff Years: .4

Human Research: Neither human cells nor tissues

Keywords: Streptococcus mitis group gene sequencing, bacterial identification

Summary: We have submitted an initiative to the National Institute for Allergy and Infectious Diseases for the development of a library of genetic sequences for critical gene targets for bacterial agents of bioterrorism and other closely related organisms. These gene targets include 16S ribosomal RNA genes and essential housekeeping genes (HKG). As a proof of this approach, we have selected viridans group streptococci because they have long been difficult to identify by traditional phenotypic methods. The viridans streptococci are a group of gram-positive bacteria that constitute part of the resident flora in the human oral cavity and gastrointestinal tract. They can occasionally be found as a cause of transient bacteremia, especially following dental procedures. This group of streptococci has become one of the important causative agents of subacute bacterial endocarditis, which can result in serious damage to heart valves. Viridans streptococci are composed of at least 22 species, which are currently divided into five subgroups: mitis, anginosus, mutans, salivarius, and bovis. Mitis group streptococci are the most common viridans streptococci responsible for diseases in humans. In the past decades, with the emergence of molecular-based methods, reclassification of viridans streptococci has been evolving and is sometimes confusing. One of the reasons for the ambiguity is that conventional biochemical tests used to identify most of the bacteria isolated in the clinical laboratory often do not provide definitive identification at the species level, and may also misidentify members of this group. A new approach for the identification of bacterial pathogens using 16S rDNA gene sequences has been recently introduced. However, this technique has not been helpful in some closely related organisms that have nearly identical sequences in their 16S genes. We have begun exploring a new set of selected HKG that may show a significant number of DNA base differences among members of the viridans streptococci, specifically the mitis group organisms. In comparison to 16S rDNA sequencing, these HKG may result in a better identification system for these organisms.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010327-01

LBC: DLM

Title: Evaluation of Microscopic Stains for Spore-forming Bacteria

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Patrick R. Murray, PhD (MICRO, CC)

Collaborator, Lab: Alexandra T. Wong (MICRO, CC)

Total Staff Years: .02

Human Research: Neither human cells nor tissues

Keywords: Spore stain, malachite green, bacillus, *clostridium*

Summary: Identification of aerobic and anaerobic gram-positive rod-shaped bacteria is frequently difficult. One important differential test is the detection of endospores in organisms such as *Bacillus* and *Clostridium*. Whereas some organisms sporulate freely and most organisms form endospores when the culture is old or maintained in unfavorable growth conditions, the detection of endospores may be difficult in relatively young cultures. For this reason, a variety of specific microscopic stains have been developed and used with varying degrees of success. In this study we initially compared two stains: a hot malachite green stain (requires heating the slide) and a cold malachite green stain. Representative isolates from the genera *Bacillus*, *Paenibacillus*, and *Clostridium* were grown overnight in culture and then slides were prepared for staining. Each of the two spore stains was performed and then compared. The hot malachite green stain was found to be superior for all organisms evaluated. More spores appeared to take up the stain, and the contrast between spores and vegetative bacteria was greater. Although the hot malachite green procedure has superior staining properties and is faster than the cold malachite green procedure, aerosol formation during the staining procedure is a potential laboratory safety concern, particularly if the culture preparation is with a highly dangerous organism such as *Bacillus anthracis*. For this reason, we are exploring modifications of the cold malachite green stain to improve the staining characteristics. One such modification is substitution of the fluorescent dye, auramine, for malachite green. A preliminary experiment demonstrated excellent staining with this fluorescent stain, offering the advantages of good contrast between spores and vegetative cells as well as decreased preparation time (less than 5 minutes are required to prepare the stained slide). We will extend this experiment to compare the fluorescent stain with the malachite green stains.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010328-01

LBC: DLM

Title: Microbial Identification using Surface-enhanced Laser Description/Ionization

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Patrick R. Murray, PhD (MICRO, CC)

Collaborators, Lab: Steven H. Fischer, MD, PhD (MICRO, CC)
Frida Stock, BS (MICRO, CC)

Collaborator, Extramural: Gerard T. Hoehn, PhD
(Ciphergen Biosystems, Inc.)

Total Staff Years: .2

Human Research: Neither human cells nor tissues

Keywords: Surface-enhanced laser desorption/ionization, bacterial identification, proteomics

Summary: Identification of bacteria has traditionally been by morphologic features and phenotypic testing. Identification of some common organisms (e.g., *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli*) can be rapid (e.g., less than 1 hour), exploiting their characteristic morphology and a few selected phenotypic tests. However, the identification of most organisms is slow, requiring hours to days for a definitive answer. More recently the use of genomics such as sequencing ribosomal RNA genes has proved to be a useful tool. Although sequencing specific genes is a powerful discriminatory tool, the current methodology requires 1 or more days before a result is available. A logical extension of sequencing genes is to use the gene products for bacterial identification. Significant variations in a structural gene sequence would result in variations in the protein product. In the past 5 years, preliminary work in the analysis of bacterial proteins for identification has been performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF MS). These studies demonstrated that whole microorganisms can provide distinct and reproducible mass spectra of proteins. The advantage of this approach is that the results are available in minutes. However, MALDI-ToF MS produces extremely complex spectra of low molecular weight proteins, which may be unsuitable for applications in the clinical microbiology laboratory. During the past 6 months, we have explored an alternative method, surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-ToF MS). The advantage of this technique is ease of use, relative low cost for the instrumentation and consumable supplies, and the use of "protein chips" for selective capture of proteins from the bacterial cell lysate. The protein chips are available in a variety of chromatographic surfaces that allow binding proteins with different affinities (e.g., cationic, anionic, and metal affinity). Bacterial lysates can be exposed to one or more protein chips and then the bound proteins analyzed by SELDI-ToF MS. In our preliminary experiments we have (1) defined the conditions required for lysing both gram-positive and gram-negative bacteria and (2) identified the appropriate protein chips for analysis. We believe we have completed the first phase of the experiments and are currently assessing the reproducibility of the protein spectra

and the discriminatory power of the profiles. If these results appear satisfactory, we will expand the study to include a large number of well-characterized, clinically significant organisms selected from our culture collection and the American Type Cell Culture (ATCC) collection. Current work on another project (CL010326-01 DLM) has revealed that phenotypic identification of culture collection isolates (including the ATCC collection) is not precise. That is, genomic studies have demonstrated that a number of collection isolates are misidentified by phenotypic tests. Therefore, the identification of all isolates used in these studies will be by comprehensive phenotypic and genotypic methods.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010330-01

LBC: DLM

Title: Validation of Rapid Sterility Test Method for Cellular Therapy Products

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Patrick R. Murray, PhD (MICRO, CC)

Collaborator, Lab: Steven H. Fischer, MD, PhD (MICRO, CC)

Collaborators, NIH: Charlie Carter, BS (CC)
Hanh Khuu, MD (CC)
Elizabeth Read, MD (CC)

Total Staff Years: .1

Human Research: Human cells or tissues

Keywords: Cell therapy products, sterility tests, BacT/Alert, bactec

Summary: Sterility testing is an essential part of in-process and release testing for cellular therapy products. The Food and Drug Administration (FDA) specifically recommends that sterility testing be performed as outlined in 21 CFR 610.12. Furthermore, because it is concerned that antibiotics may interfere with the accurate assessment of sterility testing, the FDA requires preliminary bacteriostasis and fungistasis testing according to the U.S. Pharmacopeia (USP) "Sterility Test" on all samples containing antibiotics, including cells grown in antibiotic-containing media. The methods for sterility testing described in the CFR and USP standards were developed more than 25 years ago, are labor-intensive, and require incubation for 14 days. Since the time these methods were published, more sensitive and rapid methods have been developed for detecting microbial growth in various body fluids. These modern methods include automated systems for detecting microbial growth in blood and other normally sterile fluids. Despite the fact that many laboratories use these automated systems to assess sterility of cellular therapy products, the FDA has not sanctioned this application because there are no published data from any formal comparison of these newer methods with the CFR and USP methods. For these reasons, we have developed a validation protocol comparing the CFR and USP methods with two automated culture methods: the bioMerieux BacT/Alert system and the Becton Dickinson Bactec system. Cell products will be seeded with selected bacteria and fungi and then tested with each method. The test sensitivity and time to detect a positive culture will be assessed. The validation protocol has been submitted to the FDA for comments and we anticipate that this study will be initiated in fall 2002. As supporting data for this FDA submission, we have initiated a preliminary comparison of the CFR and USP methods with the automated bioMerieux BacT/Alert system (currently used in the NIH Microbiology Laboratory). All cellular therapy products (N = 187) prepared during August-September were tested in both systems. A total of seven products had growth detected in one or both systems: three products in the BacT/Alert system only, two products in the CFR/USP method only, and two products in both systems. The clinical significance of these positive cultures will be assessed. This preliminary study will also be used to define (1) the time to detection of a positive culture in the CFR/USP and BacT/Alert methods and (2) the incidence of false-positive culture signals (e.g., the system appears positive [cloudy; positive signal by the automated system] but no organisms are recovered in the system).

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010331-01

LBC:	DLM
Title:	Evaluation of Real-time Polymerase Chain Reaction Assay for Diagnosis of *Pneumocycstis carinii* Pneumonia Using Oral Washes
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Steven H. Fischer, MD, PhD (DLM, CC)
Supervisor of Record:	Thomas A. Fleisher, MD (DLM, CC)
Collaborators, Lab:	Charles Huber, BA (DLM, CC)
Collaborators, NIH:	Joseph A. Kovacs, MD (CCM, CC) Henry Masur, MD (CCM, CC)
Collaborators, Extramural:	Laurence Huang, MD (University of California San Francisco) Hans H. Larsen, MD (Department of Clinical Microbiology, Hvidovre Hospital)
Total Staff Years:	.2
Human Research:	Human subject research
Keywords:	Pneumocystis pneumonia diagnosis, oral washes

Summary: *Pneumocystis jiroveci* (*Pneumocycstis carinii*) is an important cause of *Pneumocycstis carinii* pneumonia (PCP) in immunocompromised individuals. The standard approach for diagnosing PCP is a microscopic examination of smears prepared from induced sputum or bronchial alveolar lavage (BAL) samples. Recently, investigators have been designing and testing polymerase chain reaction (PCR) assays for the detection of *P. jiroveci* in respiratory samples. Of particular interest is the use of PCR with oral wash samples as a means of detecting *P. jiroveci* in the respiratory tract. These noninvasive specimens could prove to be of value for use in screening tests to rule out PCP. Microscopic methods are too insensitive to be useful with oral wash samples. The increased sensitivity of the PCR method, however, generates some positive results with samples obtained from patients who are colonized or infected only at a subclinical level. A precise quantitative method could help differentiate low-level colonization from infection and, consequently, improve the clinical usefulness of PCR performed on oral washes and other respiratory samples. We have developed a rapid quantitative real-time PCR assay targeting the MSG genes of *P. jiroveci* using fluorescence resonance energy transfer detection probes for signal detection. A mimic has also been constructed for inclusion in PCR reaction tubes as an indicator of false negative reactions. The assay has a detection limit of one to five MSG gene copies per amplification reaction. Results with the real-time assay using replicate samples of cloned target material vary by 10 percent or less. A blinded retrospective study was conducted using 49 lower respiratory tract samples and 49 oral wash samples collected from 51 patients with 24 episodes of PCP and 34 episodes of other respiratory disease. The average number of *P. jiroveci* copies detected in lower respiratory tract samples and oral washes from patients with PCP was significantly higher than for PCR positive patients without PCP. The results of this preliminary study have been published. A blinded, prospective study has been conducted with collaborators at the University of

California at San Francisco (UCSF) to further evaluate the performance of the real-time PCR assay in detecting PCP. The results of this study are being compiled. A second collaborative study with UCSF is underway to attempt to detect *P. jiroveci* colonization of the upper airways of health care workers after exposure to patients with PCP.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010332-01

LBC: DLM

Title: Quantitation of Residual Tumor in Patients Undergoing Allotransplantation

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Roger Kurlander, MD (HEME, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, Lab: Elizabeth S. Chao (HEME, CC)
Kristin E. Hansmann, MS (HEME, CC)
Jodie M. Keary (HEME, CC)
Meghan Ann Shipman (HEME, CC)
Abdul Tawab (HEME, CC)

Collaborators, NIH: Austin John Barrett, MD (HB, NHLBI)
Richard W. Childs, MD (HB, NHLBI)

Total Staff Years: .28

Human Research: Human cells or tissues

Keywords: Minimal residual disease, BCR-ABL, polymerase chain reaction, CML

Summary: Most patients undergoing allotransplantation at the NIH are being treated for refractory malignancy. In some cases, especially solid tumors, therapeutic effects of transplant can be monitoring adequately using conventional radiologic and serum-based assays of marker proteins. Patients treated for hematologic malignancies, however, can achieve complete remissions by conventional clinical criteria yet retain sufficient residual disease to lead to rapid recurrence without further aggressive therapy. Sensitive polymerase chain reaction (PCR)-based assays are very useful, both in monitoring the anti-tumor efficacy of individual transplants and in deciding whether additional therapeutic maneuvers, such as donor lymphocyte infusion, are needed. Such assays have been used most extensively in managing CML, a disease characterized by a chromosomal translocation which leads to the production of a unique oncogenic protein product designated BCR-ABL. DLM/hematology has previously adapted an older endpoint assay for detecting BCR-ABL cells. This test is technically difficult and time consuming. More important, it provides no quantitative data concerning the extent of CML involvement. We are currently testing newer assays exploiting real-time PCR methods. These assays are much faster to perform, comparable in sensitivity, and capable of providing quantitative data about residual tumor load. They will give us a new ability to assess the magnitude of residual BCR-ABL disease and to detect changes in disease more rapidly after therapeutic interventions post-transplant. Building on our experience in this disease, we plan to develop comparable quantitative assays for monitoring minimal residual disease in other subsets of transplantation patients, for example using the expression of WT1 to monitor residual disease in patients with acute leukemia. The ability to monitor changes in residual malignant disease will be very useful to clinicians. It also facilitates our efforts to define the impact of donor cell chimerism and alloreactivity (which are also being monitored using an ELISPOT assay in this laboratory) on the course of malignant disease in transplant patients.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010333-01

LBC: DLM

Title: Monitoring Donor T-Cell Alloreactivity during Hematopoietic Transplantation

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Roger Kurlander, MD (HEME, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, Lab: Elizabeth S. Chao (HEME, CC)
Abdul Tawab (HEME, CC)

Collaborator, NIH: Richard W. Childs, MD (HB, NHLBI)

Total Staff Years: .55

Human Research: Human cells or tissues

Keywords: ELISPOT, interferon-gamma, alloreactivity, graft-versus-host disease, T cells

Summary: Recent studies have demonstrated the feasibility of using ELISPOT assays to measure the antigen-specific responsiveness of individual T cells taken from patients. This approach is relatively simple, reproducible, and when necessary it can be modified to yield great sensitivity. Consequently, ELISPOT assays are already being used clinically in state-of–the-art trials to quantitate T cell responses to tumor- and virus-specific vaccines. This approach to date has not been used systematically to monitor alloreactivity against minor transplantation antigens and tumor antigens in the setting of human hematopoietic stem cell transplantation. We are adapting published methods for measuring responses to defined exogenous antigens for use in monitoring T cell responses to allogeneic recipient cells. When the assay is appropriately standardized, we will use it for serial monitoring of the alloreactive activity of donor leukocytes collected before transplant. By comparing these assays with post-transplant clinical course and chimerism data, we hope to establish whether pretransplant assessment can be used to predict the clinical course after transplantation. In addition, we will serially monitor the alloreactivity of T cells obtained post-transplant. Taken together, these studies seek to identify *ex vivo* findings that may correlate with or even predict clinical findings such as donor hematopoietic and immune cell engraftment, graft-versus-host disease, and graft-versus-tumor effect.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL010334-01

LBC: DLM

Title: Quantitating Engraftment of Donor Hematopoietic Cells after Transplantation

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Roger Kurlander, MD (HEME, CC)

Supervisor of Record: Thomas A. Fleisher, MD (DLM, CC)

Collaborators, Lab: Elizabeth S. Chao (HEME, CC)
Kristin E. Hansmann, MS (HEME, CC)
Jodie M. Keary (HEME, CC)
Meghan Ann Shipman (HEME, CC)

Collaborators, NIH: Austin John Barrett, MD (HB, NHLBI)
Richard W. Childs, MD (HB, NHLBI)
Douglas Hale, MD (TAB, NIDDK)
Mitchell Horwitz (LHD, NIAID)

Total Staff Years: .3

Human Research: Human cells or tissues

Keywords: Donor chimerism, polymerase chain reaction, allotransplantation

Summary: After hematopoietic stem cell transplant from a human lymphocyte antigen-matched donor, recipient blood and marrow cells are gradually replaced by donor cells. Monitoring chimerism during this engraftment is technically demanding. With assistance from Mitchell Horwitz in the National Institute on Allergy and Infectious Diseases and Richard Childs in the National Heart, Lung and Blood Institute, using commercial reagents designed for polymerase chain reaction-based microsatellite identification, we have developed assays to detect and quantitate donor cell chimerism post-transplant. These methods can detect 1 to 5 percent donor cells. Since donor engraftment in different cell compartments may vary considerably, we typically measure chimerism in CD14/15 and CD3 cells from the peripheral blood in all transplant patients undergoing non-myeloablative transplant. The resulting information about donor cell engraftment in individual patients is extremely valuable both for clinical and investigational purposes. In particular, chimerism data are used extensively by NIH clinicians in deciding when to use immunosuppressive drugs (to prevent unwanted damage by donor cells) and when to administer donor lymphocyte infusions (to promote donor-mediated destruction of cancer or unwanted host cells). In monitoring a substantial number of patients, we can recognize several distinct patterns of engraftment. Some patients engraft both CD3 and CD14/15 cells very quickly, while others receiving similar treatment engraft one or the other cell type more slowly or not at all. These patterns imply major differences in host immune response to transplantation. With time we hope to identify more subtle features useful in predicting in advance graft-versus-host disease and graft suppression of tumor growth. Using serial host chimerism data in conjunction with *in vitro* measurements of alloreactivity (using ELISPOT-based assays described in a related project), we hope to gain mechanistic insights into the role alloreactive T cells play in engraftment. Our ultimate goal is to identify factors useful in monitoring and predicting the magnitude of graft-versus-host disease and graft-versus-tumor responses.

NURSING DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL001131-03

LBC: NURS

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Margaret F. Bevans (NURS, CC)

Supervisor of Record: Clare E. Hastings, RN, PhD (OCPCS, CC)

Collaborators, Lab: Georgia J. Cusack (NURS, CC)
Susan F. Marden, RN, PhD (NURS, CC)
Helen S. Mayberry (NURS, CC)
Priscilla V. Rivera (NURS, CC)

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

Collaborators, Extramural: Nancy Kline Leidy (MedTap International)
Karen Soeken, PhD (School of Nursing, University of Maryland)

Total Staff Years: .06

Human Research: Human subject research: Interviews

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 to 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. Seventy-eight subjects have been accrued to date. Data collection continues.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001132-03

LBC: NURS

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan F. Marden, RN, PhD (NURS, CC)

Supervisor of Record: Clare E. Hastings, RN, PhD (OCPCS, CC)

Collaborators, Lab: Rosemary E. McConnell, RN, BSN (NURS, CC)
April E. Powers, RN, BSN (NURS, CC)
Colleen M. Ternisky (NURS, CC)
Margaret M. Lloyd (NURS, CC)

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

Collaborators, Extramural: Nancy Kline Leidy, PhD (Center for Health Outcome Research, MEDTAP International)
Karen Soeken, PhD (School of Nursing, University of Maryland)

Total Staff Years: .16

Human Research: Human subject research: Interviews

Keywords: HIV infection, health-related quality of life, structured intermittent therapy, symptom distress, highly active antiretroviral therapy

Summary: Because of multi-drug 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 regimens and numerous distressing side effects that may affect quality of life (QOL). The purpose of this study was to evaluate the QOL and symptom distress in individuals receiving structured intermittent (SIT) versus continuous HAART in the treatment of HIV disease. This was a companion study to a randomized trial comparing SIT with continuous HAART. Adult HIV patients in the outpatient clinic completed questionnaires measuring QOL and symptom distress using touch screen computers at seven time points over an 88-week period. Data collection for this study was stopped because of early termination of the parent protocol. Data were analyzed on 52 subjects enrolled. Two abstracts have been accepted for presentation at national meetings. A manuscript is in development.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001133-03

LBC:	NURS
Title:	Quality of Life in Melanoma Patients Receiving Vaccine Alone or with Interleukin-2
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Susan F. Marden, RN, PhD (NURS, CC)
Supervisor of Record:	Clare E. Hastings, RN, PhD (NURS, CC)
Collaborators, Lab:	Paula M. Muehlbauer, RN, MSN (NURS, CC) Debra Parchan, RN (NURS, CC) Susan A. Gantz, RN, MS (NURS, CC)
Collaborators, NIH:	Douglas Schwartzentruber, MD (NCI) Steven Finkelstein, MD (NCI) Claudia Seip, RN (NCI)
Collaborators, Extramural:	Nancy Kline Leidy, PhD (Center for Health Outcome Research, MEDTAP International) Karen Soeken, PhD (School of Nursing, University of Maryland)
Total Staff Years:	.16
Human Research:	Human subject research: Interviews
Keywords:	Melanoma, quality of life, symptom distress, Interleukin-2 therapy

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 measuring QOL and symptom distress at three timepoints: prior to, during, and post therapy. Data will be analyzed using multivariate techniques. Eighty-three subjects have been accrued to date. Data collection and subject accrual continue.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001134-03

LBC:	NURS
Title:	Quality of Life in Patients with Heart Disease and Left Ventricular Dysfunction
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Susan F. Marden, RN, PhD (NURS, CC)
Supervisor of Record:	Clare E. Hastings, RN, PhD (OCPCS, CC)
Collaborator, Lab:	Claiborne Miller-Davis, RN, BSN (NURS, CC)
Collaborators, Extramural:	Vasken Dilsizian, MD (Cardiology, University of Maryland Medical Center) Nancy Kline Leidy, PhD (Center for Health Outcome Research, MEDTAP International)
Total Staff Years:	.12
Human Research:	Human subject research: Interviews
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 was to assess the health-related quality of life (HRQL), anginal symptoms, and symptom distress experienced by patients with chronic ischemic heart disease and left ventricular dysfunction. Specifically, the relationship between underlying cardiac condition, anginal symptoms, symptom distress, and HRQL was examined. The trend in HRQL across time versus treatment group (medical or surgical management) was also evaluated. Patients responded to questionnaires measuring HRQL, anginal symptoms, and symptom distress. Underlying cardiac condition was assessed using exercise thallium imaging parameters and positron emission tomography imaging parameter (viability). Questionnaires were administered at three time intervals over a 1-year period. Data collection was stopped because accompanying parent protocols were terminated, limiting subject accrual for this study. Data are being analyzed on 25 subjects enrolled.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001135-02

LBC: NURS

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan F. Marden, RN, PhD (NURS, CC)

Supervisor of Record: Clare E. Hastings, RN, PhD (OCPCS, CC)

Collaborator, NIH: Lameh Fananapazir, MD (NHLBI)

Total Staff Years: .35

Human Research: Human subject research: Interviews

Keywords: Health-related quality of life, technology dependency, implantable defibrillators, illness representations, 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 lifesaving technology and how these perceptions influence their health-related quality of life (HRQL). The purpose of this study is to test a theoretical model that may explain the link between attitudes toward dependency on technology and HRQL 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 HRQL. Adult subjects who have received an ICD will be asked to participate. Subjects will complete a questionnaire measuring HRQL, illness perceptions, and symptom distress via the mail. Structural equation modeling techniques will be used to analyze data. Data collection for this study continues.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001136-01

LBC: NURS

Title: Pain and Palliative Care Evaluation Study

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Gwenyth R. Wallen, RN, PhD (NURS, CC)

Supervisor of Record: Clare E. Hastings, RN, PhD (NURS, CC)

Collaborators, NIH: Deloris Koziol, PhD (OD, CC)
H. Richard Alexander, MD (NCI)
Karen Baker, RN, MSN (CC)
Ann Berger, MD (CC)
Jacques Bolle, RN, DNS (CC)
David K. Henderson, MD (OD, CC)
Donna Pereira, RN, MA (CC)
Janice M. Yates, RN, PhD (NURS APPS, CC)

Total Staff Years: .1

Human Research: Human subject research

Keywords: Advanced malignancies, patient satisfaction, self-efficacy, social support, symptom management

Summary: Despite a number of descriptive studies exploring the effectiveness of specialized pain and palliative care teams, the dearth of good evaluations with any comparative design urgently needs to be addressed. This is a randomized, repeated measures evaluation study to explore the effectiveness of the inpatient pain and palliative care service intervention. The data collected during this study include both outcomes of the intervention and patient and family perceptions of the care delivery process, including issues surrounding communication with health care providers. Patients with advanced malignancies who are currently participating in National Cancer Institute Surgery Branch protocols are asked to participate in a pain and symptoms management evaluation study. Each patient and a designated family member is asked to complete a series of questionnaires over time exploring physical, psychosocial, and emotional correlates of pain and symptom management. Data will be analyzed using multivariate statistics. Fifty-three patients have been accrued to date. Data collection and subject accrual continue.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001137-01

LBC: NURS

Title: Constipation in Pediatric Cancer Patients

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Myra Woolery-Antill, RN, MN (NURS, CC)

Supervisor of Record: Clare E. Hastings, RN, PhD (NURS, CC)

Collaborators, Lab: Gwenyth R. Wallen, RN, PhD (NURS, CC)
Barbara S. Corey, RN, MSN (NURS, CC)

Collaborators, NIH: Frank M. Balis, MD (NCI)
Ellen B. Carroll, RN, BSN (APPS, CC)
Ramzi N. Dagher, MD (POB, NCI)
Elizabeth T. Fenn, RN, BSN (APPS, CC)
Paul F. Jarosinski, PharmD (CC)
Madeline Michael, RD, MPH (CC)
Seth M. Steinberg, PhD (BDMS, NCI)
Holly R. Wieland, RN, MPH (APPS, CC)

Total Staff Years: .1

Human Research: Human subject research

Keywords: Bowel movement, children, stool, symptom management

Summary: Children with cancer are treated with complex therapies, including chemotherapy, radiation, surgical interventions, and biotherapy. Treatment with vinca alkaloids and/or narcotics combined with significant lifestyle changes secondary to the disease process can have a negative impact on the child's bowel elimination status. In trying to preserve the child's health and well being, constipation can be minimized or even prevented as an unwanted side effect of the treatments or disease condition. Despite the widespread knowledge that constipation is prevalent in oncology patients, evidence shows that cancer treatment plans often overlook constipation and reflect the lack of consensus for effective assessment, treatment, and management. The research literature provides a database for addressing particular aspects of constipation. However, few studies address all the factors that affect bowel function, and fewer still have recruited pediatric populations. The Constipation Assessment Scale (CAS) is a valid and reliable measure found to be predictive of the presence and severity of constipation in the adult population; however, it has never been tested in the pediatric population. A pilot study utilizing the CAS tool in children diagnosed with cancer is being conducted. Six participants have been accrued to date. Data collection and subject accrual continue.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL001139-01

LBC: NURS

Title: Exploring the Effectiveness of a Web-based Genetics Education Program

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Janice M. Yates, RN, PhD (NURS APPS, CC)

Supervisor of Record: Clare E. Hastings, RN, PhD (OCPCS, CC)

Collaborators, Lab: Tannia P. Cartledge, MS (NURS APPS, CC)

Collaborators, NIH: Gwenyth R. Wallen, RN, PhD (NURS, CC)
Paul Hoernes, BS (CC)
Dee Koziol, PhD (CC)
Donna Krasnewich, MD, PhD (NHGRI)
Linda A. Mccullagh, MPH (APPS, CC)
Suzan M. Parada, BSN (APPS, CC)

Total Staff Years: .2

Human Research: Human subject research

Keywords: Education effectiveness, basic genetics

Summary: The National Coalition for Health Professional Education in Genetics has identified an urgent need for basic genetics education among all health care providers. Literally every Institute has protocols that have a genetics component. To address this educational need, an NIH multidisciplinary group created "Basic Genetics for Healthcare Providers." This program combines Web-based self-educational modules with traditional lectures. In health care settings, distance learning is a tool that can reach audiences on all shifts and at their work setting. The program curriculum consists of seven interactive computer modules and a monthly lecture given by a nationally recognized genetics expert. The lecture presentations were also posted on the Web modules. An e-classroom was open for questions, discussion with other participants, and responding to the "test your understanding" exercises. Responses to this program have been overwhelmingly positive. The initial course was pilot tested with 186 health care providers from various disciplines: nursing, pharmacy, social work, dietary, and medicine. Sixty-eight percent of those enrolled also participated in a pretest-post test evaluation study. The study objectives were to test the effectiveness of this type of learning and to assess the amount of learning that took place. Preliminary data from the pretest highlight the need for genetics education in the Clinical Center: 22.8 percent of enrollees had no previous Web-based education; 41.3 percent had no previous genetics education; 11.1 percent had some type of genetics information. Further analysis of the data will include paired t-test to explore mean differences in knowledge, self-efficacy, and perceived effectiveness before and after the intervention.

LBC: NURS

Title: The Role a Meticulous Oral Hygiene Program

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Janice M. Yates, RN, PhD (NURS APPS, CC)

Supervisor of Record: Clare E. Hastings, RN, PhD (OCPCS, CC)

Collaborators, NIH: Nancy Ames, MSN (CSR)
Daniel Fedorko, PhD (APPS, CC)
Henry Masur, MD (CCM, CC)

Total Staff Years: .1

Human Research: Human subject research

Keywords: Oral hygiene, intensive care unit, respiratory infection

Summary: The role of a meticulous oral hygiene program in reducing oral assessment scores, mucosal plaque scores, colonization of dental plaque, and exposure to pathogen colonization that may lead to nosocomial respiratory infections in a selected intensive care unit (ICU) patient population. Critical care patients, especially intubated individuals, have the greatest risk of any hospitalized patient of acquiring a nosocomial respiratory infection. Research reveals that the predominant initial site of bacterial colonization is the oropharyngeal cavity. Oral colonization precedes pulmonary colonization, which ultimately leads to pneumonia. Nosocomial pneumonia causes the greatest mortality and morbidity in the ICU. Prevention of colonization at the oropharyngeal site could be an effective infection control measure. Dental plaque has been identified as a host for bacterial colonization in the mouth and has been significantly associated ($p < 0.001$) with subsequent nosocomial infections. Dental plaque can act as a reservoir for pathogens in ICU patients. Aerobic pathogens are not normally associated with dental plaque. However, poor oral hygiene and lack of mechanical elimination of the plaque begins a complex cascade of biological actions by which pathogen adhesion to mucosa and teeth substrates occurs. Additionally, neglected or insufficient mouth care is the foremost predisposing factor to oral conditions such as gingivitis, mucositis, and stomatitis, which supply additional ports of entry for pathogens. Meticulous oral hygiene is required to prevent colonization of dental plaque in ICU patients. There are only three known studies that show the type and frequency of oral hygiene required to prevent or decrease colonization and thereby reduce the incidence of nosocomial respiratory infection. This prospective randomize trial tested the effectiveness of a comprehensive and systematic oral care program to reduce the mucosal plaque scores, the oral assessment scores, and the incidence of respiratory infections in patients in two selected medical-surgical ICUs. Additionally, consistency of oral hygiene was compared between the ICU whose nursing staff followed "routine" care and the ICU whose nursing staff was given specific oral hygiene teaching by a dentist and a dental hygienist. The results of the study showed that oral care provision in the test ICU was significantly better during the critical care stay than in the control ICU ($p = 0.001$). Individualize oral care was determined from the results of oral assessment scores. The amount of microbiological inoculum was significantly lower in the test ICU ($p = 0.0094$). Beck oral assessment scores improved in the test ICU ($p = 0.0342$ on day three of the ICU stay). Twenty-three patients were enrolled. Additional analysis continues.

PHARMACY DEPARTMENT

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LBC: PHAR

Title: Influence of Multi-drug Resistance Genotype on Indinavir and Saquinavir Pharmacokinetics

Dates: from 11/01/2002 to 11/01/2002

Principal Investigator: Scott R Penzak, Pharm D (CC)

Supervisor of Record: Charles E Daniels (PHARM, CC)

Collaborator, Lab: Raul M Alfaro, MS (CC)

Collaborator, NIH: Judith Falloon, MD (CMRS, NIAID)

Total Staff Years: .5

Human Research: Human subject research

Keywords: HIV, AIDS, protease inhibitor, P-glycoprotein, antiretroviral, cytochrome P450, metabolism, genetics, pharmacogenetics

Summary: The human multi-drug resistance (MDR1) gene makes a protein called P-glycoprotein (P-gp). P-gp may limit the absorption of medications, including HIV protease inhibitors. HIV protease inhibitors are drugs used to treat people with HIV infection (the virus that causes AIDS). It is possible that the particular type of MDR1 gene that a person possesses (their genotype) influences the extent to which P-gp limits the absorption of HIV protease inhibitors. The purpose of this study is to see how MDR1 genes, which people inherit from their parents, might affect how well someone absorbs the protease inhibitors indinavir and saquinavir. This study will screen 150 healthy volunteers to determine their MDR1 genotype; 60 of these volunteers will receive saquinavir (ten doses) and indinavir (four doses) and blood will be collected afterward to see whether MDR1 genotype influences the blood levels of these HIV medicines. Study subjects will also receive a single dose of midazolam to measure the activity of a particular enzyme (CYP3A) that is involved in breaking down saquinavir and indinavir in the body. This study was sent to the IRB for initial review on September 18, 2002.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL005094-01

LBC: PHAR

Title: Influence of Low-dose Ritonavir on the Pharmacokinetics of Digoxin

Dates: from 01/09/2001 to 12/31/2002

Principal Investigator: Scott R. Penzak, PharmD (CC)

Supervisor of Record: Charles E. Daniels (PHARM, CC)

Collaborator, Lab: Raul M. Alfaro, MS (CC)

Collaborators, NIH: Judith Falloon, MD (CMRS, NIAID)
Alan T. Remaley, MD, PhD (CHEM, CC)

Total Staff Years: .5

Human Research: Human subject research

Keywords: Ritonavir, P-glycoprotein, drug interaction, digoxin, drug transport

Summary: Inhibition of the MDR1 gene product and drug transporter P-glycoprotein (P-gp) is a potential cause of drug interactions in HIV-infected patients. HIV protease inhibitors (PIs) inhibit P-gp and may increase the blood levels of co-administered medications. However, controlled studies examining the influence of PIs on P-gp transport have not been investigated in humans. To characterize the influence of the protease inhibitor ritonavir (RTV) on the pharmacokinetics of digoxin (including renal clearance), 12 healthy subjects will receive a single 0.4 mg digoxin dose before and during/after 15 days of ritonavir 200 mg twice daily. An 11-day washout period will precede RTV administration. Blood and urine will be collected and sampled for digoxin, and digoxin pharmacokinetic parameter values will be determined pre- and post-ritonavir and compared. To date, data are available for 6 subjects who have completed this study. In accordance with what was expected, RTV significantly increased the blood levels of digoxin (48 percent). All six subjects experienced an increase in digoxin exposure with RTV; this increase was marked (29 to 67 percent) in five/six subjects. Preliminary results from this investigation suggest that RTV increases the blood levels of digoxin. These results are consistent with RTV-mediated inhibition of P-gp although other drugs transporters may also be involved. P-gp modulation by protease inhibitors such as RTV may be an underrecognized mechanism by which these drugs interact with concurrent medications.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL005095-01

LBC: PHAR

Title: Valganciclovir Pharmacokinetics

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Christine E. Chamberlain (CC)

Supervisor of Record: Charles E. Daniels (PHARM, CC)

Total Staff Years: .1

Human Research: Human subject research

Keywords: Valganciclovir, ganciclovir, cytomegalovirus, kidney transplant, antiviral

Summary: This study will compare different ways of giving ganciclovir and valganciclovir to kidney or kidney and pancreas transplant recipients to determine if valganciclovir provides similar protection against cytomegalovirus (CMV) compared with conventional therapy with ganciclovir. The reason for this comparison is that valganciclovir is better absorbed and has the advantage of once-a-day oral dosing. The pharmacokinetics of this drug has been studied in patients with HIV infection and CMV infection, however it has not been studied in kidney transplant patients. CMV is a serious viral infection occurring following organ transplant that can result in significant illness or death. To date, two male kidney transplant patients (one cadaveric and one living donor transplant) have enrolled. This study consists of four phases. Each phase or drug dose has been selected to mimic ganciclovir and valganciclovir use in the kidney transplant population. The first phase consists of serial blood sampling for ganciclovir blood levels after intravenous ganciclovir. The second phase consists of serial blood sampling for ganciclovir levels following oral valganciclovir at 900mg per day. These two phases will be compared for equivalency of drug levels and exposure. The third phase consists of serial blood levels following 450mg of oral valganclovir daily. The fourth phase consists of serial blood samples after oral ganciclovir 1 gram every 8 hours. The third and fourth phases will be compared for equivalency of drug levels and exposure. At this time, one subject has completed phases 1 and 2, and the second subject has completed phases 1 through 3. Since the blood samples will be pooled and processed after seven patients have completed all 4 phases, there are no results available at this time. By characterizing the pharmacokinetics of valganciclovir in the kidney transplant population, it is hoped that appropriate dosing to prevent CMV disease and limit toxicity may be achieved. This research will also be useful as a foundation to study the pharmacokinetics of valganciclovir in transplant patients with compromised renal function.

REHABILITATION MEDICINE DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL060011-23

LBC: RM

Title: Diagnostic Capabilities of Ultrasound on the Oropharynx and Larynx

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Barbara C. Sonies, PhD (SLPS, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

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

Collaborator, NIH: Carter Van Waes, MD, PhD (HNSB, NIDCD)

Total Staff Years: .66

Human Research: Human subject research: Interviews (Minors)

Keywords: Ultrasound imaging, swallowing, speech, viscosity, head and 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. We are using three-dimensional (3D) imaging that 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 National Institute on Deafness and Other Communication Disorders and National Cancer Institute 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 normal controls revealed 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 effect swallowing kinematics. We have been using 3D ultrasound imaging procedures to track post-surgical oral-facial swelling in patients who have had removal of the third molar and find that this technique is a reliable marker for change in oral facial muscles.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060017-12

LBC: PDB

Title: A Rigid Body Database on Human Movement

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Steven J. Stanhope, PhD (BS, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

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

Total Staff Years: .24

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 understand the influence of disease on function and to predict the onset of physical disability.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060042-15

LBC: RM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Barbara C. Sonies, PhD (SLPS, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

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

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

Total Staff Years: .5

Human Research: Human subject research: Interviews (Minors)

Keywords: Swallowing, neurological conditions, dysphagia, videofluorography, ultrasound imaging

Summary: We are currently analyzing the swallowing data from a group of patients with Sydenham's chorea who have been followed over the course of their recovery. We are using both ultrasound and videofluorography to examine effects of disease severity and time since appearance of chorea symptoms on swallowing and oral motor function. Patients were seen for baseline and follow-up evaluations where ultrasound and videofluorographic swallowing studies were administered along with complete oral motor function examinations. Data on swallowing performance of 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 age 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. A subset of patients with cystinosis who were seen in the late 1980s and 1990s is being re-evaluated. The effects of cystagon and kidney transplantation in this group will be evaluated. To date, of the ten returning subjects, it appears that 50 percent have not developed dysphagia or clinically observable changes in oral motor function.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060051-06

LBC: RM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan C. Robertson (OTS, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

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 National Rehabilitation Hospital revealed four occupational therapy process variables: occupational form and 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 underway.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060052-05

LBC: RM

Title: Rehabilitation Medicine Department Screening Protocol

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Lynn H. Gerber, MD (RMD, CC)

Total Staff Years: .3

Human Research: Human subject research

Keywords: Functional measures

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 may resume optimal performance in their daily living activities. The RMD Screening protocol is designed to allow RMD staff members the opportunity to pilot new tests, techniques, therapeutic modalities, technology, or equipment that has very low or no risk. These tests/techniques may be commonly used in rehabilitation practice but are being tested in a population that is different from the traditional. This protocol is designed for pilot work, data from which are used to generate a protocol when appropriate or to ensure investigators of the ease/usefulness of the assessment, technology, or equipment. The RMD Screening protocol was used to pilot the following projects: (1) adapting the Biodex System 2 to assess the isometric peak force and endurance of patients with inflammatory muscle disease; (2) piloting assessments planned for the Phase II National Cancer Institute Clinical Trial of BMS-247550, epothilone B analog, in patients with breast carcinoma; and (3) pilot study to test instruments to be used in the neurotoxicity protocol.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060054-02

LBC: RM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Barbara C. Sonies, PhD (RMD, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

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

Collaborator, Extramural: Christian Macedonia, MD (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, with 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 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 86 fetuses (GA 15 weeks to 39 weeks) in women ages 19 to 42 and will continue until we have 100 normal-appearing fetuses and 20 that appear to be at risk for developing delayed feeding patterns.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060055-02

LBC:	RM
Title:	Task-induced Physiological and Biomechanical Changes of the *In vivo* Human Tongue
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Gloria Chi-Fishman, PhD (RMD, CC)
Supervisor of Record:	Lynn H. Gerber, MD (RMD, CC)
Collaborators, Lab:	Jeri L. Miller, PhD (RMD, CC) Barbara C. Sonies, PhD (RMD, CC)
Collaborators, NIH:	Alan S. Barnett, PhD (NIMH) John A. Butman, MD (CC) A. Scott Chesnick (NHLBI) Betty Wang, PhD (CC)
Collaborators, Extramural:	Lucinda A. Pfalzer, PhD (Physical Therapy, The University of Michigan – Flint)
Total Staff Years:	.24
Human Research:	Human subject research
Keywords:	Tongue, *in vivo*, human, volumetrics, hemodynamics, magnetic resonance imaging, ultrasound, diffusion tensor

Summary: The overall objective of this protocol is to better understand the normal physiological responses of the tongue to contraction tasks. Specifically, our goals are to (1) quantify three-dimensional volumetric changes of the tongue as a function of maximal voluntary contraction tasks, (2) examine task-induced changes in blood flow and how tongue vessels and muscles interact during graded lingual contractions, and (3) determine task-induced variations in the diffusion properties of water molecules in lingual tissue. We have recruited a total of 25 healthy volunteers, with whom we conducted on-site tongue task training and magnetic resonance imaging (MRI) screening. Eighteen of these individuals qualified; 15 were studied between 11/23/01 and 8/26/02, and three will be studied after 10/7/02. Experimental tasks included resting (R) and bolus-holding (H) postures, and oral (O) and oropharyngeal (P) maximum voluntary isometric contractions. From the MRI data, we have computed and normalized the mean difference scores in tongue volume by subject across tasks. No apparent differences were observed for H-R and O-R. Statistical analysis of P-R (range = 5.42-10.06 percent difference, M = 7.98, SD = 1.77) and P-H (Range = 4.20-11.76 percent, M = 7.69, SD = 2.19) with alpha at .025 showed significant difference from a hypothetical mean of zero ($p<.0001$). Intra-subject variations in calculated volume for each task were small (<1 percent), reflecting high subject consistency and intra-rater reproducibility. Preliminary analysis of regional volume changes in the lingual vascular bed in two subjects also showed significant task-induced differences. Analysis of data on lingual blood flow waveform (velocity, resistive index, etc.) and inner vessel diameters during post-contraction reperfusion as a

function of task and by vessel site revealed: (1) similar baseline arterial diameters ($p=0.542$) at all recording sites (main, sub-, and deep lingual arteries); (2) consistent increase in vessel diameters at start of reperfusion ($p<0.001$), but no difference in diameter across contraction regions ($p=0.106$); and (3) increase in volume flow following both Oral and Orop contractions ($p = 0.002$) and across all arterial sites ($p=0.002$) with main lingual artery having the highest reperfusion volume ($p=0.005$). This MVIC profile differed from that of dry swallows in reperfusion rate, return-to-baseline rate, volume flow, and velocity, but not in vessel diameter.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060056-02

LBC: RM

Title: Effect of Task on Oral Pressure Dynamics during Swallowing

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Gloria Chi-Fishman, PhD (RMD, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

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

Collaborators, NIH: Jean-Pierre Guadagnini, DDS (NIDCR)
Robert A. Wesley, PhD (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/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 who cannot benefit from sequential swallowing as a compensatory strategy. We have studied 14 healthy volunteers and four patients with neurologic impairments. Our measurements included total duration (TD) of pressure bulb activation, peak pressure distribution ratios [(time to peak mmHg)/TD and (peak-to-end time)/TD], peak mmHg, start-to-peak and peak-to-end slopes, and area under curve. Significant main effects of Task ($p<.0124$) and Bulb ($p<.0001$) were found with no significant interaction. Further task comparisons for each bulb with Tukey adjustments of p attributed most of the significant contrasts to the posterior bulb. The most salient findings were: for RSeq swallows, peak pressure was reached proportionally 1.5-fold farther into the swallow than for discrete tasks, and the rate of pressure change from peak to baseline was twice as rapid. RSeq swallows also differed in bulb activation pattern. For example, two subjects showed an "on and partial off" pattern for anterior and mid bulbs; in four other subjects, these bulbs (or the anterior alone) were not activated for selected RSeq swallows. In addition, we found: (1) considerable intra- and inter-subject variability across all tasks, but relatively more so for discrete than for RSeq swallows; (2) no striking task-based difference in peak pressure; and (3) a trend for RSeq swallows to be shorter in TD (thus smaller in area under curve). Our preliminary findings suggest task-induced differences in pressure distribution strategy and a more efficient way of swallowing for RSeq by delaying the peak, thus devoting a greater portion of the swallow to bolus propulsion. This supports our thesis of the potential clinical application of rapid sequential swallowing in selected patient populations.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060057-01

LBC: RM

Title: Morbidity following the Diagnosis and Treatment of Patients with Breast Cancer

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Charles L. Mcgarvey (PTS, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

Collaborators, Extramural: Cindy Pfalzer, PhD (Physical Therapy, University of Michigan)
Peter Soballe, MD (Breast Care Center, National Naval Medical Center)

Total Staff Years: .2

Human Research: Human subject research

Keywords: Morbidity, breast cancer, lymphedema

Summary: This retrospective (case-control) outcome study will investigate the frequency and severity of morbidities in a population of approximately 165 patients diagnosed with breast cancer before and after medical and surgical treatment. The study will be conducted between two sites, the Warren G. Magnuson Clinical Center and the National Naval Medical Center in Bethesda, Maryland. Subjects will be followed for 1 year with quarterly examinations (baseline [pre-medical treatment] and at 1, 3, 6, 9, and 12 months after treatment). In order for cancer survivors to understand the risk of impairment and functional limitations and disability; and for health care providers to determine the risk of physical impairment, functional limitations, and loss of independence (morbidity) in patients with breast cancer, it is necessary to study these patients from the point of diagnosis (before surgery) to a reasonable period following the completion of the primary treatment program (1 year after medical treatment). Although pain, numbness, fatigue, lymphedema, and diminished physical function are described as prevalent and debilitating conditions, remarkably few clinical studies are published describing the associated physical impairments, functional limitations, or methods for their control with measures prior to medical intervention and long-term followup. The proposed outcome study will include 1) a retrospective review of specific medical record information such as staging conference information and the standard clinical quarterly examination during a 1-year period and 2) administration of a follow-up outcome questionnaire, a physical activity questionnaire, and a quality-of-life questionnaire at the 6- and 12-month time points. The outcome survey is an upper limb disability questionnaire developed as an outcome measure for this project. Data available in these measurement domains will allow the researchers to determine 1) the frequency and severity of a) symptom distress (fatigue, pain including chronic pain, aching, weakness, burning, tingling, numbness, anxiety, and depression) and pathological conditions (adhesive capsulitis, weakness and atrophy, neuropathy, scar/skin adhesions, lymphedema), b) physical impairments (diminished upper extremity and trunk range of motion/flexibility, strength, coordination, and increased girth), and c) functional limitations and disabilities during the course of the medical treatment (loss of independence in or ability to perform routine activities of daily living, i.e., grooming, bathing, dressing, driving an automobile and, in some cases, return to their regular work, recreational, and social activities) and 2) level of impairment at which these patients have lost independence in function and identity of patients at higher risk for the loss of independence in function (e.g., activities of daily living).

Purpose: To examine the frequency and severity of problems in women with breast cancer during the first year after initial medical treatment, including physical impairments, such as loss of strength or flexibility, increased weight, and swelling; symptom distress, such as pain, fatigue, and weakness; functional limitations and disabilities, such as loss of independence in activities of daily living (e.g., grooming, bathing, dressing, driving a car), work, and social and recreational activities; and to identify factors associated with these problems and try to determine their relationship to them. This study will correlate the frequency and severity of impairment with demographics, characteristics of tumor/stage of disease, and treatment-related (surgery, chemotherapy, and radiation therapy) factors.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL060058-01

LBC: RM

Title: Morbidity following Breast Cancer Treatment: A Prospective Study

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Charles L. Mcgarvey (PT S, CC)

Supervisor of Record: Lynn H. Gerber, MD (RMD, CC)

Collaborators, Extramural: Lucinda A. Pfalzer, PhD (Physical Therapy, The University of Michigan – Flint)
Peter Soballe, MD (Breast Care Center, National Naval Medical Center)

Total Staff Years: .2

Human Research: Human subject research

Keywords: Morbidity, breast cancer, lymphedema

Summary: This longitudinal, prospective outcome study will describe the frequency and severity of morbidities and investigate the risk factors for development of morbidity defined as upper limb impairments, functional limitations and disability in a treatment group of approximately 160 patients diagnosed with breast cancer before and after medical and surgical treatment compared to a control group of 160 women who undergo excisional breast biopsy to rule out breast cancer with negative findings. Subjects will be followed for 2 years with periodic examinations (baseline [pre-surgical/medical treatment] and at 1, 3, 6, 12, 18 and 24 months after treatment). In order for cancer survivors to understand the risk of impairment and functional limitations, and disability; and for health care providers to determine the risk of physical impairment, functional limitations, and loss of independence (morbidity) in patients with breast cancer; it is necessary to study these patients from the point of diagnosis (before surgery) to a reasonable period following the completion of the primary treatment program (2 year after medical treatment). The proposed outcome study will include: 1) a two year longitudinal, prospective design that includes a control group; 2) specific patient process variables such as demographics, medical data, e.g., staging conference information, and the standard upper body clinical examination; and 3) administration of a self-report surveys/questionnaire that measure upper limb functional limitations and disabilities, physical activity and quality of life at baseline and follow-up at 1, 3, 6, 12, 18 and 24 months. Data available in these measurement domains will allow the researchers to determine the: 1) frequency and severity of: a) symptom distress (fatigue, pain including chronic pain, aching, weakness, burning, tingling, numbness, anxiety, and depression) and pathological conditions (adhesive capsulitis, weakness and atrophy, neuropathy, scar/skin adhesions, lymphedema), b) physical impairments (diminished upper extremity and trunk range of motion/flexibility, strength, coordination and increased girth), and c) functional imitations and disabilities during the course of the medical treatment (loss of independence in or ability to perform routine activities of daily living, i.e., grooming, bathing, dressing, driving an automobile, and in some cases, return to their regular work, recreational and social activities). 2) level of impairment at which these patients have lost independence

in function and identity those patients at higher risk for the loss of independence in function (e.g., ADLs), and 3) risk factors for loss of function and disability. Purpose: to examine the frequency and severity of problems in women with breast cancer for 2 years following initial medical treatment, including: Physical impairments, such as loss of strength or flexibility, increased weight and swelling; Symptom distress, such as pain, fatigue and weakness; and Functional limitations and disabilities, such as loss of independence in activities of daily living (e.g., grooming, bathing, dressing, driving a car), work and social and recreational activities. To identify factors associated with these problems and try to determine their relationship to them.

TRANSFUSION MEDICINE DEPARTMENT

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INTRAMURAL RESEARCH PROJECT 1 Z01 CL002040-18

LBC: DTM

Title: Significance of Anti-HIV Antibody in Asymptomatic Donors

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Harvey J. Alter, MD (IDS, CC)

Collaborators, Lab: Cathy C. Conry-Cantilena, MD (IDS, CC)
Susan Leitman, MD (IDS, CC)
Cathy Schechterly, BA (IDS, CC)

Total Staff Years: 1.2

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 follow-up 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 sexual partners, and 8 percent 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 males 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 have exceeded 10 years of follow up and 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 loads to CD4 counts and outcome. HIV coreceptors will be measured as indicated. No evidence of HIV infection evolved in the initial anti-HIV positive but WB-indeterminate or WB-negative subjects. Treatment with highly active antiretroviral therapy is being conducted by personal physicians or through other NIH protocols.

LBC: DTM

Title: Etiology of Allergic Reactions in Platelet and Granulocytapheresis Donors

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborator, Extramural: Kearby Fugate, PhD (CDRH, FDA)

Total Staff Years: .1

Human Research: Human cells or tissues

Keywords: Allergic reactions, platelet and granulocytapheresis donors

Summary: In February 1984, the Department of Transfusion Medicine 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 (RASTs), and basophil histamine release assays, specific IgE-mediated sensitization to ethylene oxide (EO), a gas used to sterilize the plastic disposable apheresis kits, was found in 10 of 16 plateletpheresis donors and 8 of 10 granulocytapheresis donors experiencing reactions, but in none of 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 the Food and Drug Administration (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 it 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 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 will also be tested, as will a large sample of samples derived from the National Health and Nutritional Examination Survey study.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002062-12

LBC: DTM

Title: Treatment of Familial Hypercholesterolemia by Dextran Sulfate Apheresis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

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

Collaborator, Extramural: Deno Zachary, ScB
(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 high-density lipoprotein 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 10 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 to 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 Food and Drug Administration for treatment of FH in July 1996. Patients are now continuing long-term follow-up 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. One of them is likely to be the oldest survivor in the world with this disorder, and has undergone biweekly apheresis therapy for the past 25 years at the NIH.

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James Waikuo Shih, PhD (RMD, CC)

Collaborators, NIH: Harvey J. Alter, MD (DTM, CC)
Richard Y. Wang (DTM, CC)

Total Staff Years: .9

Human Research: Human cells or tissues

Keywords: Viral genomes

Summary: There are two components in this project; both are extended from our continuous commitment to the clinical investigation of viral hepatitis. One 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 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 hepatitis B virus, hepatitis C virus (HCV), and human immune deficiency virus infection. Whenever possible, we would improve the basic PCR technique to make it a semi-quantitative procedure. During the past 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 viral agents that may be responsible for hepatitis cases with unidentifiable cause. Due to its great resource requirement, we tried to conduct this project with industry partners under collaborative research and development agreements. 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.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002068-11

LBC:	DTM
Title:	A Prospective Study of Anti-hepatitis C Virus-positive Blood Donors
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Harvey J. Alter, MD (IDS, CC)
Collaborators, Lab:	Cathy C. Conry-Cantilena, MD (IDS, CC) Susan Leitman, MD (IDS, CC) Cathy Schechterly, BA (IDS, CC) James Waikuo Shih, PhD (IDS, CC)
Collaborator, NIH:	Jay Hoofnagle, MD (DDN, NIDDK)
Collaborators, Extramural:	Joan Gibble, MD (American Red Cross, Chesapeake) Paul Ness, MD (Department of Lab Medicine, Johns Hopkins University)
Total Staff Years:	1.2
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+ 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. The 10-year experience with this cohort is being analyzed for publication.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002071-07

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborator, Lab: Maria P. Bettinotti (LAB SS, CC)

Collaborator, NIH: Franco Marincola, MD (CC)

Total Staff Years: .7

Human Research: Human subject research

Keywords: Human leukocyte Antigen, immunogenetics, vaccination, target cycle reaction, polymorphism

Summary: Human leukocyte antigen (HLA) genes are the most polymorphic genes in the human genome. Knowledge of HLA polymorphism in relation to possible peptide-based, T cell-restricted vaccination protocols is important for understanding the physiology of T cell recognition and improving strategies of T-cell antigen-specific vaccination. During the past 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 antigen identification program based on co-transfection of HLA and cDNA libraries into permissive antigen presenting cells (in this fashion a new epitope for anti-cancer 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; and (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 by 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.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002076-07

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James Waikuo Shih, PhD (RMD, CC)

Collaborators, NIH: Harvey J. Alter, MD (DTM, CC)
Qu Qui, MD (DTM, CC)
Richard Y. Wang (DTM, CC)

Total Staff Years: .3

Human Research: Neither human cells nor tissues

Keywords: Nucleic acid vaccine, hepatitis C, hepatitis C virus

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. This long-term project can be globally subdivided into four phases. The initial phase is design and construction of immunogenic plasmids. This includes continued monitoring the development of the understanding of HCV immunity and selecting the most beneficial gene delivery systems. The second phase is to determine the immunogenicity of these constructs. During this step, we are experimenting with gene delivery strategy and possible use of adjuvants or other immune modifiers. The third phase is to evaluate the immune responses, and the final phase is to conduct the protectivity study. Great efforts have been devoted to determine the immune response, including assay developments. The protectivity study would have to be conducted in an appropriate animal model. The optimal challenge inoculums for HCV vaccine remain to be determined. Each of these phases would have many different components, and the development, implementation, evaluation, and improvement steps could be overlapping. In FY 95 and 96, 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 of 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 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 non-structural 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 years, 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. More recently, we have conducted studies combining several immunogens to evaluate their interaction or interference. We also developed a boost strategy. We are in the process of evaluating our prime boost procedures and formulations. 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 10/01/2001 to 09/30/2002

Principal Investigator: Harvey J. Alter, MD (IDS, CC)

Collaborators, Lab: Cathy Schechterly, BA (IDS, CC)
James Waikuo Shih, PhD (IDS, CC)

Collaborators, NIH: Barbara Rehermann, MD (LD, DDB, NIDDK)
Robert Purcell, MD (LID, NIAID)

Collaborator, Extramural: Patrizia Farci, MD
(Department of Medicine, University of Calgiari)

Total Staff Years: .9

Human Research: Human cells or tissues

Keywords: Hepatitis C, quasi-species, HCV T cell immunity

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 rapidly recover, those who have delayed recovery, those with persistent infection and stable chronic disease, and those with rapidly progressive, fatal infection. The parameters measured will be 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 T cell helper, proliferative, and cytotoxic responses. The goal is to determine if any of these parameters can predict outcome. Studies to date have shown no correlation with genotype since 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* 2000, 288:339-344). 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. Thus far, studies have shown that patients with chronic HCV infection have impaired CD4 and CD8 cell responses to all HCV antigen functions compared with patients who recover from acute HCV infection.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002079-06

LBC:	DTM
Title:	Hepatitis C Virus Infection in Infants and Children
Dates:	from 10/01/2001 to 09/30/2002
Principal Investigator:	Harvey J. Alter, MD (IDS, CC)
Collaborators, Lab:	Cathy A. Schechterly (IDS, CC)
Collaborators, Extramural:	Camilla Baxter, BS (Hematology, Childrens National Medical Center) Naomi Luban, MD (Hematology, Childrens National Medical Center) Parvathi Mohan, MD (Gastroenterology, Childrens National Medical Center)
Total Staff Years:	.2
Human Research:	Human subject research: Minors
Keywords:	Hepatitis C, hepatitis C in infants and children, pediatric HCV

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 follow-up 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. A total of 5,546 children who met eligibility criteria were transfused at CNMC during this interval. 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) HGV RNA positive. The HCV and HGV prevalence in age-matched nontransfused 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 a mean of 24 months. All are asymptomatic. The range of alanine aminotransferase (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 follow-up with serial liver biopsies is necessary before the true disease burden can be ascertained. This study will have major implications for anti-viral 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 CL002080-07

LBC: DTM

Title: Natural History of Hepatitis C Virus Infection

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Harvey J. Alter, MD (IDS, CC)

Collaborator, NIH: Leonard Seeff (DDP, NIDDK)

Total Staff Years: .3

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 follow-up 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 follow-up 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 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 follow-up 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.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002082-07

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Harvey J. Alter, MD (IDS, CC)

Collaborator, NIH: Barbara Rehermann, MD (LD, DDB, NIDDK)

Collaborators, Extramural: Michael Busch, MD, PhD (Department of Transfusion Medicine, Blood Centers of the Pacific)
Krishna Murthy, DVM (Virology/Immunology, Southwest Foundation for Biomedical Research)

Total Staff Years: .05

Human Research: Human subject research

Keywords: Viral hepatitis, AIDS, chimpanzee, HCV, HIV

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 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. In contrast to the HIV experiment, we have found that HCV can be transmitted by blood that has levels of HCV RNA undetectable by the most sensitive nucleic acid testing assays currently available for blood screening. 2) In collaboration with Cerus Corp., the chimp model was used to establish the efficacy of psoralen/ultraviolet (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 follow-up, 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.

LBC: DTM

Title: Development of Methods for *ex vivo* Cultured and Immunologically and/or Genetically Modified Cells

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Elizabeth J. Read, MD (CELL, CC)

Supervisor of Record: Harvey Klein, MD (CC)

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

Collaborators, NIH: Harry L. Malech, MD (LHD, NIAID)
A. John Barrett, MD (NHLBI)
Fabio Candotti, MD (NHGRI)
Daniel H. Fowler (MB, NCI)
Ronald E. Gress, PhD (EIB, NCI)
Jennifer Puck, MD (NHGRI)
Scott Solomon, MD (HB, NHLBI)

Total Staff Years: .3

Human Research: Human 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, as follows: Preparation of allogeneic donor lymphocytes selectively depleted for alloreactive T cells using an anti-CD25 immunotoxin (collaboration with the National Heart, Lung and Blood Institute, Stem Cell Transplant Program): In FY 2001, we completed development and scale-up of this complex process, and in September 2001, initiated a phase I clinical trial of DLI selectively depleted of donor-specific alloreactivity in the setting of allogeneic hematopoietic transplantation. In FY 2002, three patients were entered on the clinical trial and received their transplants plus SD cells. This year, we also made plans to evaluate two cell manipulation strategies as alternatives to the immunotoxin: one, an immunomagnetic separation method and the other, ultraviolet light-activated killing of cells after incubation with a photosensitizing agent. Preparation of donor Th2 T cells for clinical trials (collaboration with the National Cancer Institute): 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 27 patients have been treated through FY 2002. 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; that study was completed in 2001. At the end of FY 2001, 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, and two patients have been treated. In FY 2002, methods were improved (with new cytokines) and validated for gene transduction of autologous PBSC and bone marrow of patients with X-linked severe combined immunodeficiency, and this clinical trial will begin pending resolution of scientific and Food and Drug Administration investigation of the child with apparent vector-induced oncogenesis.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002085-07

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Elizabeth J. Read, MD (CELL, CC)

Supervisor of Record: Harvey Klein, MD (CC)

Collaborators, Lab: Charles S. Carter (CELL, CC)
Hanh Khuu, MD (CELL, CC)

Collaborators, NIH: Harry L. Malech, MD (LHD, NIAID)
Austin John Barrett, MD (HB, NHLBI)
Ronald E. Gress, PhD (EIB, NCI)
Robert J. Lederman, MD (VBB, NHLBI)

Total Staff Years: .6

Human Research: Human 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 that have developed systems for potential application to clinical cellular therapies: 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 versus 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*). Nexell, Inc., Isolex: Studies of the automated Isolex 300i for immunomagnetic selection of hematopoietic progenitor cells were completed. More than 100 selection procedures on version 2.0 (either positive only or combined positive/negative selection) have been completed, and more than 100 selection procedures on version 2.5 were completed over the past 3 years. Studies of combined positive and negative selection aimed at achieving 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. Miltenyi CliniMacs/CD34 positive selection: In FY 2000, we performed a preclinical study of positive selection of normal donor mobilized PBSC using this system. 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. Miltenyi CliniMacs/AC133 positive selection: In FY 2002, we initiated a preclinical study, in collaboration with the National Heart, Lung and Blood Institute Cardiology Branch, on selection of peripheral blood cells positive for AC133, a newer marker of progenitor cells that includes the angioblastic lineage. Two selection procedures have been done to date. This study will be continued into FY 2003 and will serve as the foundation for clinical trials of *ex vivo*-generated angioblasts for treatment of coronary artery and myocardial disease.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002089-06

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborator, Extramural: Jaime Oblitas, MT (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 10e10 cells with dexamethasone alone versus 4.87 and 7.31 x 10e10 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 had restlessness, insomnia, bone pain, or headache. Ten percent of donors requested discontinuation of participation in the study due to the inconvenience and discomfort of the mobilization regimen. Fifty-six Clinical Center patients have received G-CSF mobilized granulocytes. Thirty-five were profoundly neutropenic, including 15 patients with severe aplastic anemia (SAA), 12 stem cell transplant recipients, seven patients with lymphoma/leukemia, and one with breast cancer. The remaining 21 patients had CGD. In the neutropenic patients, 21 had systemic filamentous fungal infections, 11 had bacterial infections, two had candidemia, and one had RSV infection. The mean increment in granulocyte count 1 hour post-transfusion was 2,600/uL, and counts greater than 500/uL above baseline were sustained for 12 to 24 hours. One of the 14 neutropenic, immunosuppressed patients who survived longer than 2 weeks after the initiation of granulocyte transfusions developed human lymphocyte antigen (HLA) allosensitization, as did two of the 15 CGD patients. In the absence of HLA allosensitization, granulocyte transfusions were associated with progressive hypoxia, pulmonary infiltrates, and an adult respiratory distress syndrome-like event in four of 15 SAA patients, versus one of 21 CGD patients. Of the neutropenic patients with tissue molds, 10 of 21 stabilized or improved during granulocyte transfusion therapy, but only five of 21 survived hospitalization. In contrast, five of 11 with bacterial processes were discharged from hospital. Eighteen of 21 patients with CGD had resolution of their fungal (nine of 12) or bacterial (nine of nine) 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 multicenter study of the efficacy of G-CSF mobilized granulocyte transfusions in severely neutropenic patients with filamentous fungal infections is being organized by the Hemostasis/Transfusion Medicine Clinical Trials Network of the National Heart, Lung, and Blood Institute.

LBC: DTM

Title: Peripheral Blood Stem Cell Collections from National Marrow Donors Program Donors

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborators, Extramural: Robyn Ashton, RN (Marrow Donor Center, DTM, NIH)
Dennis Confer, MD (Chief Medical Officer, NMDP)

Total Staff Years: .3

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 August 31, 2002, 4.8 million donors were participating in the registry and 14,859 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 that 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 62,000 donors on its registry, is participating in a nationwide NMDP protocol for the acquisition of filgrastim-stimulated PBSCs by apheresis of unrelated donors. 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 versus PBSC donation; and (3) to monitor the outcome of matched unrelated-donor PBSC transplants, including time to engraftment, incidence of graft-versus-host disease (GVHD), and disease-free and overall survival. As of September 30, 2002, 61 NIH donors had undergone 79 apheresis procedures to collect PBSCs for unrelated NMDP recipients. Forty-four of 61 (72 percent) required only a single apheresis procedure to collect an adequate cell dose for transplant. Seventeen of 61 had a poor CD34 mobilization response to filgrastim and needed two consecutive apheresis procedures to collect an adequate cell dose. Three of the 61 donors, all female, required a central line. All donors experienced granulocyte-colony stimulating factor (G-CSF)-induced fatigue, insomnia, bone pain, or headache, although in only 8 percent were these effects considered severe. Peak mean leukocyte counts after filgrastim were 45,500/uL, and postapheresis thrombocytopenia (less than 100,000/uL) occurred in 9 of 61 donors (15 percent), all of whom underwent two procedures. The mean time to complete recovery from PBSC donation was 1 week, compared with 3 weeks for marrow harvest. Ten of 15 donors who had donated both marrow and PBSC preferred G-CSF-stimulated apheresis donations to marrow harvest due to the lack of need for anesthesia and hospitalization; discomfort

of the two procedures was considered equivalent. Analysis of NMDP recipient outcomes shows that PBSC transplants are associated with reduced times to engraftment and improved acute transplant-related morbidity compared with marrow transplants. However, GVHD incidence and severity are increased with PBSC versus marrow grafts, so that overall survival at 1 year is not different between the two types of unrelated transplants. Administrative and statistical support for this study is 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 10/01/2001 to 09/30/2002

Principal Investigator: David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborators, Lab: Maria P. Bettinotti (LAB SS, CC)
Lorraine G. Caruccio (LAB SS, CC)

Total Staff Years: 1.3

Human Research: Human subject research

Keywords: Granulocyte antigens

Summary: 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. Neutrophil-specific antigen HNA-2a (NB1) has been localized to CD177 glycoprotein (gp), which is expressed on subpopulations of neutrophils. PRV-1 is a gene that is overexpressed in neutrophils from patients with polycythemia rubra vera. The gene encoding NB1 differs from PRV-1 at four reported nucleotides. The goal of this study was to determine if PRV-1 and NB1 were alleles of the same gene or two separate genes, and if they are alleles of the same gene, to determine the gene frequencies of each allele and explore potential correlations to neutrophil CD177 gp expression. Primer pairs were used to amplify leukocyte genomic DNA in the regions surrounding the four NB1 polymorphic sites within exon 1, 3, 8, and 9. The four resulting amplicons were sequenced and analyzed for each donor. The size of the neutrophil population in each donor staining brightly with CD177 antibody was assessed by flow cytometry. If PRV-1 and NB1 are separate genes, then all people tested should be heterozygous for the PRV-1/NB1 polymorphisms. Since six of 16 donors tested were homozygous for PRV-1 polymorphisms at all for sites, PRV-1 and NB1 are alleles of the same gene, CD177. When the sequenced exons in the 16 donors were compared to PRV-1, 14 single-nucleotid polymorphisms (SNPs) were found. Thirteen of the 14 SNPs result in amino acid changes. The G42C exon 1 NB1 polymorphisms was the most common SNP. It was found in seven donors. The seven SNPs in exons 1, 2, and 3 are in the CD177 gene, but it is uncertain if the seven SNPs in exons 7, 8, and 9 are in CD177 or a homologous pseudo gene. Thirty-two SNPs were also found in introns 2, 7, and 8. Since the G42C SNP resulted in an amino acid change in the CD177 protein leader sequence, the size of the CD177 bright neutrophil population was compared among donors homozygous of G at bp42, 42GG, and those homozygous for C, 42CC. The CD177 bright neutrophil population was greater in 42CC donors than in 42GG donors. These studies show that PRV-1 and NB1 are alleles of the polymorphic gene CD177. The most common SNP in bp42 predicted an amino acid change in the protein's leader sequence and effects protein expression. Future studies will be directed toward evaluating the entire CD177 gene sequence in normal donors and in patients with polycythemia vera.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002097-04

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Elizabeth J. Read, MD (CELL, CC)

Supervisor of Record: Harvey Klein, MD (CC)

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

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

Total Staff Years: 1

Human Research: Human 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 FY 1999, 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. In FY 2000, 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 was published in January 2002. In FY 2001, we 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. During FY 2002, we completed studies demonstrating stability after overnight hold at 4°C of the raw material (mononuclear cell concentrates) in terms of ability to generate immature and mature DCs. We also completed stability testing of mature peptide-pulsed DCs and established a 2-hour room temperature hold period to accommodate transport or delays in administration of the product. In addition, we established a program to document lot-to-lot potency of the CD40 ligand reagent used to mature DCs in culture. Ongoing studies are focused on characterizing DCs by flow cytometric phenotyping.

LBC: DTM

Title: Use of Granulocyte Colony Stimulating Factor in Healthy Donors

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborator, Lab: Susan Leitman, MD (DTM, CC)

Total Staff Years: .1

Human Research: Human subject research

Keywords: Granulocyte colony Stimulating factor, granulocyte concentrates, peripheral blood stem cells

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 prior to donation, generally 12 to 24 hours before the start of apheresis. G-CSF has also been given for 5 days to hematopoietic stem cell donors prior collecting peripheral blood stem cell (PBSC) concentrates by apheresis. PBSC concentrate donors given G-CSF experience splenic enlargement and rarely, spontaneous rupture of the spleen. This study evaluated the incidence and time course of splenic enlargement in PBSC concentrate donors and accessed factors effecting size changes. Twenty healthy adult PBSC concentrate donors were given G-CSF (10 mg/kg/day) for 5 days. Ultrasound was used to assess craniocaudal spleen length prior to giving G-CSF, the day of apheresis, and 3 or 4 days after apheresis. The effects of donor age, gender, race, and changes in blood chemistries, blood counts, and CD34+ cell counts on spleen length change were assessed. Spleen length increased in 19 of 20 donors. Mean length changed from 10.7 cm pre-G-CSF to 12.3 cm on the apheresis day ($p < 0.002$). Three or four days after apheresis the spleen length fell to 11.3 cm ($p<0.001$), but remained greater than baseline levels ($p=0.04$). There was no difference in spleen length change among males and females or among Caucasians and non-Caucasians. There was no relationship between subject age and change in length or percent change in length. There was no relationship between baseline blood counts and chemistries and change in apheresis day size but apheresis day alkaline phosphatase and total bilirubin levels were related to change in length. These studies found that spleen size increases in almost all PBSC donors. Enlargement is transient but marked in some donors and may place donors at risk for splenic rupture. Greater collection day alkaline phosphatase, bilrubin and neutrophil levels were associated with greater increases in spleen size. Future studies will focus on identifying factors that predict which donor will have a very large increase in spleen size.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002101-04

LBC: DTM

Title: Plasma Exchange Donation in Treatment of von Willebrand Disease

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: von Villebrand disease, apheresis, cryoprecipitate

Summary: Von Willebrand 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. Characterization of the child's bleeding disorder revealed a FVIII:C level of 4 percent, FVIII:Ag level of 20 percent, vWF:RCo of 21 percent, vWF:Ag 3 percent, indicative of severe vWD. The child's father carried an allele with a defect at the level of vWF mRNA expression, but he 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 cryosupernatant plasma as replacement fluid. During the first 15 years of the patient's life, the father underwent 55 plasma exchange donations, yielding a total of 150,633 units of FVIII. 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 -70°C. Ninety-two percent of the cryoprecipitate was transfused after 1 year of storage, with a mean collection to transfusion interval of 2 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 15, 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 10/01/2001 to 09/30/2002

Principal Investigator: David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

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 falciparum* 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 brisk to be accounted for by the destruction of *P. falciparum*-infected red blood cells (RBCs), since 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* infection also induces 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. In collaboration with Captain Trever Jones of the U.S. Naval Medical Research Center, we have been investigating malaria anemia in an aotus monkey model. We have found that monkeys vaccinated with *P. falciparum* protein EBA-175 and challenged with low levels of parasites became severely anemic despite level 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. Further investigations of malaria anemia using this model are underway.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002103-03

LBC: DTM

Title: Immune Therapy for Cytomegalovirus Infection

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborators, Lab: Maria P. Bettinotti (LAB SS, CC)
Jong Baeck Lim (LAB SS, CC)

Collaborator, NIH: A. John Barrett, MD (NHLBI)

Total Staff Years: 1.4

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 pneumonitis, 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 cytotoxic 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 immunotherapy and to better understand the cellular immune response to CMV. Current studies are focused on identifying the immune-dominant 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 immune-dominant pp65 peptides for people with types HLA-A*0101 and HLA-A*0301. We have also begun collaborative studies with Dr. John Barrett, National Heart, Lung and Blood Institute. He is planning to vaccinate hematopoietic stem cell donors with a canary pox vector containing the gene for CMV proteins PP65 and IEP prior to the collection and transplantation of stem cells. We will be monitoring the donors' immune response to the vaccination.

LBC: DTM

Title: Molecular Testing Standards and New Amplification Approaches

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James Waikuo Shih, PhD (RMD, CC)

Collaborators, NIH: Richard Y. Wang (DTM, CC)

Total Staff Years: .2

Human Research: Neither human cells nor tissues

Keywords: Molecular testing standards

Summary: There are two components within this program. 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 collaborative research and development agreement (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 hepatitis C virus (HCV) internal standard (IS) based on murine amphotropic retrovirus. To achieve this, we went through a stepwise process including mutating the 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 and pending jointly by the University of Maryland and NIH based on these observations. 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. We are continuing our effort to find a manufacturer for licensing purposes.

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: James Waikuo Shih, PhD (RMD, CC)

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

Collaborator, Extramural: Shyhching Lo, PhD, MD
(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 AFIP to investigate the co-factors 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 diseases (STDs) 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 ODS. Thus, no serological evidence suggests that 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.

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. A polymerase chain reaction (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. We are continuing our collaboration but limiting our effort to the advising and technical support level.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002107-03

LBC: DTM

Title: Prospective Studies of Phlebotomy Therapy in Hereditary Hemochromatosis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

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

Total Staff Years: .3

Human Research: Human 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 pre-set therapeutic endpoints were reached. We enrolled 113 patients with HH in the first 2 years 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 percent below pretreatment baseline. A fingerstick 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. Median pretreatment values in the first 53 previously untreated patients included ferritin 1,035 (range 26 to 4,938) ng/mL, transferrin saturation (TS) 72 (29 to 106) percent, and MCV 95.6 (84 to 105) cubic microns. Mean ferritin was 27 ng/mL, TS was 22 percent, and HGB was 13.7 g/dL at the point of transition from induction to maintenance therapy, as defined by the MCV guide. A mean of 25 induction bleeds were performed in HFE homozygotes and 14 bleeds in C282Y/H63D double heterozygotes until iron depletion was achieved. Mean nadir HGB of 13.3 g/dL occurred 2 (0 to 4) weeks after the transition to maintenance therapy, and mean nadir MCV of 87.4 occurred 6 to 8 weeks after the transition. 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 double 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 9 to 10 weeks for C282Y/C282Y homozygotes, and every 11 to 12 weeks for compound heterozygotes 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. Eighty-five (75 percent) of the HH subjects met donor eligibility criteria. The contribution of red cell units derived from HH subjects to total allogeneic inventory in our center has progressively increased to, and now stabilized at, 9 percent of all allogeneic red cell units collected at our center. Positive viral markers were found in four 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. Universal genetic screening for HH, and use of HH donor blood for transfusion (from otherwise eligible donors) would increase the number of blood units collected in the United States by 12 percent and alleviate periodic critical blood shortages.

LBC: DTM

Title: Studies of ABO Incompatibility in Hematopoietic Stem Cell Transplantation

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

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

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Peripheral blood stem cell transplantation, hemolysis, ABO incompatibility, pure red cell aplasia

Summary: The ABO blood group system is critically important in blood transfusion. However, ABO incompatibility has had less dramatic impact in hematopoietic transplantation. Since ABO and human leukocyte antigens (HLA) are inherited independently, ABO incompatibility may occur in up to 20 to 40 percent of HLA-matched allogeneic hematopoietic stem cell transplants (SCT). Immune hemolysis caused by donor "passenger lymphocytes" contained in the stem cell graft, and pure red cell aplasia (PRCA) associated with prolonged production of recipient type antidonor isohemagglutinins, have each been described with varying frequency after marrow-derived SCT performed with myeloablative conditioning when there is minor or major ABO incompatibility, respectively, between donor and recipient. However, despite the occurrence of these events, the overall impact of ABO incompatibility in SCT is generally considered low, providing that appropriate transfusion practices are followed. The introduction of peripheral blood stem cells (PBSC) as the hematopoietic graft source, and the use of low-intensity nonmyeloablative regimens for recipient conditioning, have dramatically widened the applications of SCT. PBSC are supplanting bone marrow as the preferred stem cell source due to more rapid hematopoietic recovery, improved survival, and relative ease of collection. PBSC also contain an order of magnitude more lymphocytes than bone marrow-derived grafts. Low-intensity conditioning regimens have further reduced regimen-related toxicity and rely on the generation of graft-versus-tumor immune effects rather than drug- or radiation-induced cytotoxic effects to eradicate malignant disease. The combination of PBSC and reduced-intensity conditioning is also increasingly being utilized for older patients and for those with debilitating transfusion-dependent non-malignant hematologic diseases. In conjunction with investigators in the National Heart, Lung, and Blood Institute, National Cancer Institute, and National Institute of Allergy and Infectious Diseases, the Department of Transfusion Medicine (DTM) in the Warren Magnuson Clinical Center at NIH has prospectively evaluated the impact of ABO incompatibility following SCT when utilizing PBSC grafts and either conventional myeloablative or reduced-intensity non-myeloablative conditioning. We observed massive immune hemolysis in three of ten consecutive patients undergoing HLA-identical, related-donor PBSC transplants with minor ABO incompatibility. Nonablative conditioning was used in nine of these ten cases, including two with hemolysis,

while cyclosporine alone was used as prophylaxis against graft-versus-host disease (GVHD) in 10/10. Catastrophic hemolysis of 78 percent of the circulating red cell mass led to anoxic death in the first case, but severe consequences were avoided by early, vigorous donor-compatible red cell transfusions in the subsequent two cases. Hemolysis began 7 to 11 days after PBSC infusion. A prophylactic 10-unit red cell exchange performed in one patient reduced the circulating recipient-type red cell mass by only 60 percent; therefore red cell exchanges were not performed in subsequent cases. All patients with hemolysis had a positive direct antiglobulin test (DAT), with eluate reactivity against the relevant recipient antigen. However, neither the intensity of the DAT, the donor isohemagglutinin titer, nor other factors could reliably be used to predict the occurrence of hemolysis. Because immune hemolysis with PBSC grafts has been observed following both ablative and non-ablative conditioning, the increased incidence and severity of hemolysis appears to be most closely related to the PBSC graft lymphocyte content, and use of CsA without an antiproliferative agent for GVHD prophylaxis. Hemolysis has not been reported using PBSC grafts in which the lymphocyte content is depleted by *ex vivo* processing. The DTM continues to evaluate the incidence of hemolysis in this setting, including recent protocols in which mycophenolate mofetil or methotrexate have been added to CsA in the anti-GVHD regimen. To evaluate the effect of major ABO incompatibility on donor red cell engraftment and PRCA following reduced-intensity non-myeloablative SCT (NST), we compared consecutive series of patients with major ABO-incompatible NST (fludarabine/cyclophosphamide conditioning) and myeloablative SCT (cyclophosphamide/high-dose TBI). Because substantial host hematopoiesis may persist following NST, and hematopoietic and immune function may be both host and donor in origin (mixed chimerism) for prolonged periods, the kinetics of donor erythropoiesis might be expected to differ substantially after major ABO-incompatible immune-based NST compared with traditional myeloablative SCT. We found that donor red blood cell (RBC) chimerism (initial detection of donor RBC in peripheral blood) was markedly delayed following NST versus myeloablative SCT, median 114 versus 40 days, and strongly correlated with decreasing host anti-donor isohemagglutinin levels. Anti-donor isohemagglutinins declined to clinically insignificant levels more slowly following NST than myeloablative SCT (median 83 versus 44 days). Donor RBC chimerism was delayed more than 100 days in nine of 14 (64 percent) and PRCA occurred in four of 14 (29 percent) patients following NST, while neither event occurred in 12 patients following myeloablative SCT. PRCA lasted 123 to 220 days, and patients with PRCA required a mean of 27 red cell units in the absence of other reasons for transfusion support. Conversion to full donor myeloid chimerism following NST occurred significantly sooner in cases with, compared to those without, PRCA (30 versus 98 days). Patients with a delayed onset of donor red cell chimerism who did not develop PRCA had a delayed conversion to full donor myeloid chimerism, and were protected from reticulocytopenia by a bridge of autologous erythropoiesis. Cyclosporine withdrawal appeared to induce graft-mediated immune effects against recipient isohemagglutinin-producing cells, resulting in decreased anti-donor isohemagglutinin levels and resolution of PRCA following NST. These data indicate that hemolysis may be frequent and severe after transplantation of minor ABO-incompatible PBSCs when utilizing cyclosporine alone to prevent GVHD. We recommend that meticulous clinical monitoring and early, vigorous donor-compatible red cell transfusions be practiced in all such instances. The data also indicate that significantly delayed donor erythropoiesis may be common following major ABO-incompatible NST and is associated with prolonged persistence of host anti-donor isohemagglutinins. The clinical manifestations of these events are affected by the degree and duration of residual host hematopoiesis, and may be especially significant when utilizing reduced-intensity conditioning to treat patients with poor pre-transplant erythropoietic function such as aplastic anemia, sickle cell disease, and thalassemia.

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002110-02

LBC: DTM

Title: Transfusion-Related Infections Prospectively Studied (TRIPS)

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Harvey J. Alter, MD (IDS, CC)

Collaborators, Lab: Mary Helen Boone (IDS, CC)
Tamica Cain (IDS, CC)
Pamela L. Hernandez (IDS, CC)
Harvey Klein, MD (IDS, CC)
Cathy A. Schechterly (IDS, CC)
James Waikuo Shih, PhD (IDS, CC)
Bernice L. Williams (IDS, CC)

Collaborator, NIH: T. Cain, BA (CC)

Collaborator, Extramural: Michael Busch, MD, PhD (Department Transfusion Medicine, Blood Centers of the Pacific)

Total Staff Years: 2.9

Human Research: Human subject research Human 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 over the safety of the 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 a candidate hepatitis virus, HGV; 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- 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 existence 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, the repository would allow future testing for prions in new variant Creutzfeld-Jacob disease (human variant of mad cow disease) or 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 CL002111-02

LBC: DTM

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

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborator, Lab: Susan Leitman, MD (DTM, CC)

Collaborator, NIH: Alan N. Schechter, MD (LCB, NIDDK)

Total Staff Years: .1

Human Research: Human subject research

Keywords: Sickle cell trait, red blood cells, leukocyte reduction filters

Summary: Fifty to seven-five percent of red blood cell (RBC) components from donors with sickle cell trait occlude leukocyte reduction filters. People with sickle cell trait are healthy, but very low oxygen levels, low pH, and high hemoglobin concentrations can induce RBC 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 are responsible for the failure of RBC components from donors with sickle cell trait to filter. 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. 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. Since adding 60 mL of air effectively increased oxygen levels, units of blood from 10 sickle cell trait donors were divided into two. Half was placed into a bag with 60 mL of air and the other into a bag without air. Both halves were incubated for 2 hours. RBC components were prepared and passed through leukocyte reduction filters. We found that when sickle trait donor blood (n = 10) was incubated with 60 mL of air, nine of ten components filtered completely. Only one of nine components incubated without air filtered completely. These studies show that RBC components from sickle cell trait donors can be successfully filtered when oxygen levels are increased. Current studies are focused on developing a method to increase oxygen levels in stored blood that is suitable for use with blood that will be transfused.

LBC: DTM

Title: Citrate Effects and IV Magnesium during Large Volume Leukapheresis

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborators, Lab: Charles Bolan, MD (DTM, CC)
Salim A. Haddad, MD (DTM, CC)
Yu Ying C. Yau, RN (DTM, CC)

Total Staff Years: .7

Human Research: Human cells or tissues

Keywords: Leukapheresis, apheresis, magnesium, hypomagnesemia, calcium, hypocalcemia, citrate

Summary: Large volume leukapheresis (LVL) is defined as processing greater than three blood volumes, or greater than 15 liters of whole blood in an adult, during a single leukapheresis (white blood cell collection) procedure. LVL is increasingly being used to harvest peripheral blood stem cells (PBSC) and other mononuclear cells (MNC) for hematopoietic transplantation and immune reconstitution. Decreases in divalent cation levels caused by administration of citrate anticoagulant during LVL can be associated with severe donor reactions, and may limit the rate at which blood can be processed. Several donors at NIH experienced citrate-related hypocalcemic tetany during LVL, in response to which we developed standard operating procedures for the routine administration of prophylactic intravenous calcium solutions during longer apheresis procedures. Other centers have used heparin to reduce the amount of citrate given to the donor during the procedure; however, this exposes the donor to systemic anticoagulation and may be associated with hematomas or clumping of the apheresis product. We previously determined that citrate administration during apheresis was associated with a marked increase in urinary excretion of calcium and magnesium during the procedure, and that performance of prolonged or repeated LVL caused rapid and significant decreases in blood calcium and magnesium levels. Decreases in calcium levels were ameliorated during procedures performed with prophylactic administration of intravenous calcium solutions. Our preliminary studies also demonstrated that ionized magnesium levels markedly declined during LVL. The clinical impact of severe, acute decreases in ionized magnesium levels in healthy apheresis donors was not clear, however. Since most of the adverse effects related to citrate administration can be prevented by prophylactic calcium administration, it is unknown what aspect of the remaining discomfort may be attributable to hypomagnesemia. This protocol will focus on determining the contribution of acute hypomagnesemia to citrate-related symptoms during large volume apheresis, and establishing the role of and indications for prophylactic intravenous magnesium replacement in this setting. The study plan will consist of a prospective, randomized, placebo-controlled, double-blind study. Healthy allogeneic PBSC or MNC donors will be assigned to one of two treatment groups. One group will receive intravenous magnesium infusions throughout all scheduled LVL procedures; the other group will receive an

infusion of an equivalent volume of intravenous normal saline as placebo. Symptom scores and blood samples will be obtained by apheresis nurses at periodic sampling intervals. Laboratory assays will be performed by associate laboratory investigators in a blinded fashion. To date, five subjects have accrued to the study and undergone a total of nine LVL procedures using intravenous magnesium or saline infusions. The treatment assignment code has not been broken, and thus the symptom scores and laboratory data have not yet been evaluated. Fifty-two subjects will be studied, 26 in each group (magnesium replacement versus saline placebo).

INTRAMURAL RESEARCH PROJECT 1 Z01 CL002113-01

LBC: DTM

Title: Plasmapheresis of Anthrax Vaccinees for Production of Anthrax-immune Globulin

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborator, Lab: Bonnie L. Sink, RN (DTM, CC)

Collaborators, Extramural: Nina Marano, DVM, MPH (Meningitis and Special Pathogens Branch, NCID/CDC)
Philip Pittman, MD (USAMRIID, DOD)

Total Staff Years: .2

Human Research: Human cells or tissues

Keywords: Anthrax, vaccine, plasmapheresis, immune globulin

Summary: Inhalational anthrax infection is associated with a 60 to 100 percent mortality rate, depending on the rapidity with which appropriate antimicrobial therapy is initiated. Experiments using an animal model of inhalational anthrax suggest that adjunctive therapy with equine-derived, anti-anthrax antisera may be associated with higher survival rates; however, no human-derived antisera are currently available. The purpose of this protocol is to provide a mechanism for obtaining high-titer, anti-anthrax immunoglobulin by plasmapheresis of human volunteers who have recently received a course of anthrax vaccination. Volunteers are Department of Defense (DoD) employees and military personnel who are within 3 to 12 weeks of having received a fourth or greater dose of AVA if four to six total inoculations were given, or within 6 months of the last dose if seven or more AVA inoculations were given. All vaccinees were vaccinated as a requirement of their tour of duty and will otherwise meet all blood donor eligibility criteria, in accord with Food and Drug Administration (FDA) requirements and American Association of Blood Banks (AABB) standards. Plasmapheresis will be accomplished using licensed apheresis devices and standard collection techniques, and products will meet all blood safety testing requirements currently mandated by the FDA. Plasma components collected under this protocol will be stored in the frozen state as fresh frozen plasma (FFP) until a protocol for administration of these components to human patients critically ill with inhalational anthrax infection has been reviewed and approved by the Institutional Review Boards of NIH, the Centers for Disease Control and Prevention (CDC), and U.S. Army Medical Research Institute of Infectious Diseases. Following this approval, products will undergo standard Cohn-Oncley fractionation into a concentrated immunoglobulin preparation suitable for intravenous use, to be designated anthrax immune globulin intravenous (AIGIV). Intravenous administration of products derived from plasma collected under this protocol, whether as single-donor FFP or as AIGIV, will occur under a Phase I/II trial involving an Investigational New Drug (IND) exemption, with the IND held by the CDC.

The plasma products collected under this protocol will also be used in pharmacokinetic, dose finding, and efficacy studies in animals, and to establish a repository of reference serum standards at the CDC. To date, three AVA vaccinees have undergone four to six plasmapheresis donations each. From these donations, a total of 36 bags of Anthrax Immune Plasma (AIP), constituting 12 therapeutic doses of AIP, have been prepared. These AIP components are in long-term frozen storage, available for immediate release, if clinically needed. Serum anti-PA (anthrax protective antigen) titers in the donors did not change with serial weekly plasmapheresis. This study represents a collaborative effort between the Department of Transfusion Medicine/NIH, CDC, and DOD.

LBC: DTM

Title: Irradiated Sporozoite Vaccination to Prevent Malaria

Dates: from 10/01/2001 to 09/30/2002

Principal Investigator: Susan Leitman, MD (DTM, CC)

Supervisor of Record: Harvey Klein, MD (DTM, CC)

Collaborator, Extramural: Thomas Richie, MD (Malaria Program, NMRC)

Total Staff Years: .1

Human Research: Human cells or tissues

Keywords: Malaria, irradiated sporozoite, vaccine, plasmodium, apheresis

Summary: The purpose of this protocol is to provide a mechanism for the Department of Transfusion Medicine, Clinical Center, to perform leukapheresis procedures on subjects participating in an experimental irradiated sporozoite vaccination program for the prevention of plasmodium (malaria) infection, conducted by the Malaria Program at the Naval Medical Research Center (NMRC). The leukapheresis procedures are necessary to enable collection of sufficient numbers of cells for in vitro laboratory studies to follow the effects of the vaccine. The maximum number of subjects who will undergo leukapheresis is 60: 30 vaccinees and 30 nonvaccinated controls over the course of 10 years, with each subject undergoing apheresis on four occasions. The involvement of NIH in this study is the limited to the performance of leukapheresis procedures. The study design, subject recruitment, vaccine development, subject vaccination, laboratory monitoring, and data analysis are performed by NMRC staff, and all study participants must be active-duty employees of the Department of Defense. To date, a total of 29 study subjects have undergone 66 leukapheresis procedures. The procedures consisted of discontinuous flow procedures performed using a Haemonetics MCS Plus device, and processing four "passes" per procedure. Mean (+ SD) volume processed was 2,155 ± 171 mL, with a mean product yield of 4.34 ± 0.77 x 109 total white cells, 82 percent of which were mononuclear cells (65 percent lymphocytes, 17 percent monocytes). Specific cells yields included 2.84 ± 0.59 x 109 lymphocytes and 0.76 ± 0.29 x 109 monocytes. The products also contained a mean of 14 mL of packed red cells and 1.92 x 1011 platelets. No significant adverse effects of apheresis were noted.

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