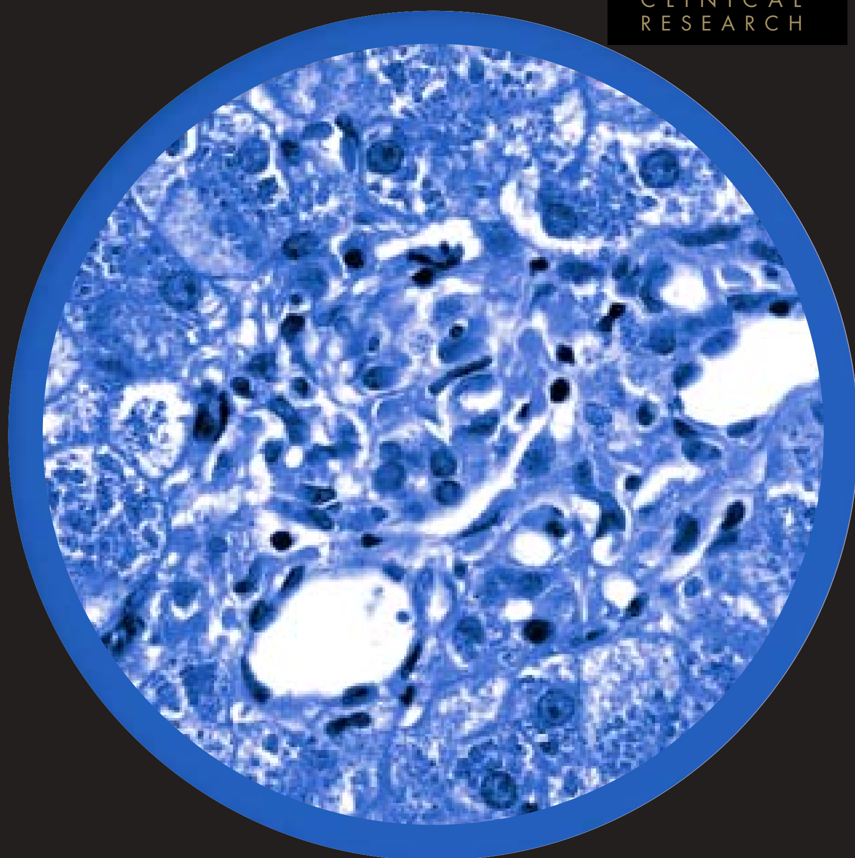
Warren G. Magnuson Clinical Center



Annual Report of Clinical Research Activities

OCTOBER 1, 2002 TO SEPTEMBER 30, 2003

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH

Cover art:
stain of hepatocytes, liver cells, in a patient with excess iron.

Annual Report of Clinical Research Activities

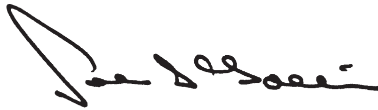
OCTOBER 1, 2002 TO SEPTEMBER 30, 2003

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

2003 marked the commemoration of the NIH Clinical Center's 50th anniversary. During this historic year, Clinical Center researchers and staff continued their ongoing commitment to excellence in biomedical research. Clinical Center departments supported new and expanding areas of science including molecular imaging techniques, pharmacology, immune cell harvesting, and advances in biomechanics.

In addition to facilitating the biomedical research efforts of colleagues in institute programs, Clinical Center researchers continue to be recognized for their scientific accomplishments. As we prepare for the opening of the Mark O. Hatfield Clinical Research Center and activating the new Clinical Research Information System next year, Clinical Center investigators will continue to support institute research initiatives while conducting exemplary independent research activities. This report summarizes clinical research projects overseen by Clinical Center investigators in FY 2003.



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

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Z01 CL008047-02

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Zenaide Quezado, MD (CC)

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

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

Total Staff Years .21

Human Research Neither human cells nor tissues

Keywords sepsis, peritonitis, infection, nitric oxide, NOS1, nNOS, mice, knock out

Summary Several studies have now 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 called 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 knockout [KO]) have increased leukocyte rolling and adhesion to the endothelium of postcapillary venules and increased leukocyte migration into the peritoneal cavity. The purpose of our 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 have conducted initial experiments in our mouse model of peritonitis and sepsis, and demonstrated that, although NOS1-KO animals have reported increased migration of neutrophils in the setting of chemical peritonitis, in the setting of live bacterial peritonitis, genetic deficiency of NOS1 is detrimental and decreases survival. In addition, we found that 7-nitroindazole, a specific inhibitor of NOS1, had opposite effects in wild-type animals compared with NOS1-KO. Future studies will be designed to elucidate the mechanism whereby NOS1 affects survival, leukocyte trafficking, and bacterial clearance during sepsis and septic shock.

Z01 CL008056-01

Title Development of a Noninjurious Neuro-Specific Nociceptive Assay in Mice

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Zenaide Quezado, MD (CC)

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

Total Staff Years .1

Human Research Neither human cells nor tissues

Keywords pain, mouse, neurometer, and nociception

Summary Pain is the most common reason why patients seek health care and usually is the consequence of tissue damage that occurs during or after medical illness, surgery, or trauma, including chemical and thermal injuries. More recently, pain has been regarded as a fifth vital sign along with blood pressure, heart rate, temperature, and respirations. Fortunately, the understanding of the neurophysiologic mechanisms by which noxious and nonnoxious stimuli are perceived has improved substantially over the last few years, and new drugs and techniques have been developed to treat pain. However, the mechanisms of pain are not entirely understood, and humans still have a great deal of pain while in the hospital. In fact, many people have debilitating chronic pain throughout their lives. The purpose of this study is to establish a noninjurious neuro-specific nociceptive assay in mice. To this end, we are using a Food and Drug Administration-approved neuro-stimulator that is used in humans for the diagnosis of pain syndromes and neurologic diseases. This device delivers electrical stimuli to the skin at different frequencies and intensities, and produces transient discomfort without producing injury. Given that currently used pain models have limitations and may produce some tissue injury in order to induce pain, the development of a noninjury-producing animal model would be an invaluable tool for the study of the mechanisms of pain. With this study, we have used a technique that does not stimulate the peripheral cutaneous nerve organ used for the transmission of a painful stimulus (nociceptors) and does not produce tissue injury, but does produce transient discomfort and pain-avoiding behavior by stimulating specific nerve fibers. Under DASS 02-01, we completed pilot studies for the development of a noninjurious neuro-specific nociceptive assay in mice, developed normative values for the response to each frequency, and developed fully a software application to control the neuro-stimulator and automate a large portion of the protocol. The automation also required the development of custom hardware, which is also used to digitally record sensor signals and events. Subsequent investigations will further validate the model and demonstrate its value for studies of the mechanisms of pain and pharmacodynamics of new therapeutic agents to treat pain.

Clinical Bioethics Department

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Z01 CL010505-05

Title	Patient Perspectives on Health Insurance
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Marion Danis, MD (CBD, CC)
Supervisor of Record	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborators, Extramural	Richard Duke, PhD (Richard Duke & Associates) Susan Dorr Goold, MD, MSHA, MA (University of Michigan) Charles Hall (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) use this tool to examine how patients enrolled in managed care organizations would choose to define their health insurance benefit package. The study instrument has been designed and pilot tested, and the study design is complete. Study subjects were recruited and 50 group exercises have been conducted. Data collection was completed on June 23, 2000. Data have been processed and data analysis has been completed. One manuscript has been published and another is in press. The tool has been developed into a computer-based exercise by the NIH and the University of Michigan and has been copyrighted by the University of Michigan.

Z01 CL010507-05

Title	Physician Resolution of Ethical Problems
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Marion Danis, MD (CBD, CC)
Supervisor of Record	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborators, Extramural	Brian Mi Clarridge (University of Massachusetts) Gordon DuVal, SJD (University of Toronto)
Total Staff Years	.3
Human Research	Human subject research: Interviews
Keywords	ethical problems, physicians
Summary	This protocol is designed to identify the most frequent and difficult ethical problems encountered by physicians; to examine how physicians resolve these ethical problems; to examine how physicians utilize ethics consultation services; and to determine what barriers or deterrents physicians perceive in utilizing ethics consultation services. Data collection for this study has been completed. Several analyses have been completed and several are ongoing. One manuscript has been published, one is in press, one has been submitted, and one is in preparation.

Z01 CL010512-04

Title	Minnesota CHAT: Public Perspectives on Health Insurance in Minnesota
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Marion Danis, MD (CBD, CC)
Supervisor of Record	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborators, Extramural	Ellen Benavides (Health Strategies Group Andrea Biddle, PhD, MPH (UNC–Chapel Hill) Susan Dorr Goold, MD, MSHA, MA (University of Michigan)
Total Staff Years	.2
Human Research	Human subject research: Interviews
Keywords	health insurance, Minnesota CHAT

Summary

This protocol was designed to determine how residents of Minnesota would choose to define their health insurance benefit package. The results are intended to inform the managed care industry in Minnesota to attend to rising costs and dissatisfaction with choices in health care. The study instrument has been designed, and the study design is complete. Participant recruitment and participation are complete. Data processing and data analysis are complete. One manuscript has been submitted and revised for publication, and one manuscript is being prepared for publication.

Z01 CL010513-04

Title A Comparative Study of Ethical Issues in Multinational Clinical Research

Dates from: 10/01/2002 to: 09/30/2003

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 .7

Human Research Human subject research: Interviews

Keywords ethical issues, multinational clinical research

Summary This study seeks to inform deliberation and resolution of ethical issues related to multinational clinical research through interviewing various participants of the ESPRIT study. ESPRIT is a multinational collaborative clinical trial of Interleukin-2 in HIV disease. Our study is interviewing four groups participating in ESPRIT: (1) chairs of the Institutional 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 regarding important ethical issues associated with ESPRIT. There has been substantial controversy about the ethics of human subjects research in developing countries. This study is designed to provide an ethical framework for clinical research in developing countries and investigate empirically some of the more controversial issues in this research. We are still conducting interviews with IRB chairs, PIs, and research participants in several countries.

Z01 CL010514-04

Title Promises of Benefit Phase I Oncology Informed Consent Forms

Dates from: 10/01/2002 to: 09/30/2003

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 .9

Human Research Neither human cells nor tissues

Keywords informed consent

Summary This protocol will analyze consent forms for phase I oncology trials to assess how the nature, risks, and potential benefits are communicated. Phase I consent forms were collected from all of the 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 involving interviews with 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 over-promise benefit or downplay risk. In fact, only one 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 discussed risk comprehensively, including mentioning the possibility of death.

Z01 CL010515-02

Title Ethical Issues with Nurse Practitioner and Physician Assistant Practice

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Christine Grady, PhD (CBD, CC)

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

Collaborators, Lab Connie M. Ulrich, RN, PhD (CBD, CC)
Marion Danis, MD (CBD, CC)

Collaborator, NIH Deloris Koziol, PhD (OD, CC)

Total Staff Years .4

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' (nurse practitioner [NPs] and physician assistants [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 the NP and PA for the provision of quality cost-effective health care. In addition, as part of the methodological purpose of the study, to evaluate the effectiveness of monetary incentives to increase response rates to a mailed self-administered questionnaire, subjects will be assigned to one of three incentive groups randomly. 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. Surveys were mailed, beginning in November 2002, to 3,900 nonphysician clinicians in the United States. Data on ethical conflicts and impact on practice are being analyzed. Response was highest in the group randomized to a $5 cash incentive, and a manuscript on the randomized incentives has been submitted.

Z01 CL010516-02

Title Survey of Institutional Review Board Chairs Regarding Interpretation of U.S. Federal Pediatric Regulations

Dates from: 10/01/2002 to: 09/30/2003

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 .2

Human Research Human subject research: Interviews

Keywords Institutional Review Board, assent

Summary In order to assess the protection of children who are enrolled in clinical research, it is important to assess how Institutional Review Boards (IRBs) reviewing such research interpret and implement the Federal Regulations for research with children set forth in 45CFR 46 Subpart D. This study aims to gather this information through interviews with IRB chairpersons. In particular, the survey aims to assess how IRBs assess risk/benefit levels of research with children, when IRBs permit children's assent to be waived, what information IRBs require children to be present 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 National Institute of Child Health and Human Development (NICHD) funding, and chairpersons of the currently functioning private IRBs. This study was approved by the NICHD IRB. Surveying is complete and we are currently writing up the results.

Z01 CL010517-02

Title	Assessing the Assent Process in Pediatric Research
Dates	from: 10/01/2002 to: 09/30/2003
Principal Investigator	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborator, Lab	David S. Wendler, PhD (CBD, CC)
Collaborators, Extramural	Gail Geller, ScD (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	.2
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 for what information children must be given prior to soliciting their assent. Similarly, the Federal Regulations do not provide any guidance on how soliciting 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 the role of children in making decisions on their participation in clinical research. This study was approved by the National Institute of Child Health and Human Development Institutional Review Board (IRB) and the Duke University IRB. We are currently conducting pretest interviews.

Z01 CL010518-02

Title A Survey of European Physicians Regarding Ethical Dilemmas in Clinical Practice

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Marion Danis, MD (CBD, CC)

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

Collaborator, Lab Samia Hurst, MD (CBD, CC)

Collaborators, Extramural
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Reidun Forde, MD (Norwegian Medical Association)
Reidun Pegoraro Renzo, MD (Fondazione Lanza)
Arnaud Perrier, MD (Hopitaux Universitaires de Geneve)
Stella Reiter-Theil, MD (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 in situations of scarce resources. 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. The study has been amended to include 500 U.S. internists for comparison with the European sample. Institutional Review Board (IRB) approval has been attained at the NIH and in each of the participating countries for the original sample. IRB approval at the NIH for the additional U.S. sample has been obtained and Office of Management and Budget approval is being sought. Data collection is currently ongoing for the European sample and will begin for the U.S. sample when the approval process is complete.

Z01 CL010519-02

Title Perceptions of Benefits and Risks – Rakai

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Christine Grady, PhD (CBD, CC)

Supervisor of Record 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 (Uganda Virus Research Institute)
F. Nalugoda, BSc, MHS (Uganda Virus Research Institute)
D. Serwadda, MB, ChB, MMed, MPH (Makerere University)
J. Wagman, MHS (Columbia University)
Maria Wawer, MD (Columbia University)

Total Staff Years .4

Human Research Human subject research: Interviews

Keywords developing countries, multinational, ethics, survey, Africa

Summary 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 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 will assess perceptions of the effect of research on individuals and communities. The Rakai Project is an ongoing prospective cohort study in the Rakai District of southwestern Uganda. It has an intensive population-based HIV/STD epidemiological, behavioral, and intervention research program in 46 communities in the district. A representative sample of men and women was 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 by their communities. Men and women interviewed come from deep rural, rural, and peri-urban communities. A quantitative survey has been administered to approximately 800 individuals; qualitative in-depth individual interviews and focus group discussions are underway. Data from the quantitative surveys are being cleaned and readied for analysis.

Z01 CL010520-02

Title	Ethics Substudy of an Anti-Malarial Efficacy Study in Uganda
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Christine Grady, PhD (CBD, CC)
Supervisor of Record	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	.4
Human Research	Human subject research: Interviews
Keywords	informed consent, multinational, stored blood samples, ethics, survey, Africa

Summary

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 Antimalarial Treatment (EANMAT) sites in Uganda, Rwanda, Tanzania, and Kenya, and will evaluate the safety and efficacy of LapDap (chlorproguanil/dapsone) plus artesunate for uncomplicated malaria in children aged 3 months to 59 months. It will also compare the efficacy of LapDap with that of sulphadoxine pyrimethamine plus amodiaquine. The ethics substudy will be conducted at four Ugandan sites. Individual interviews have been conducted with more than 300 parents (primarily mothers) of children enrolled in the malaria study. The data have been entered and analysis is underway.

Z01 CL010521-01

Title User Evaluation of the CHAT Website

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Marion Danis, MD (CBD, CC)

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

Total Staff Years .3

Human Research Human subject research: Interviews

Keywords evaluation studies, decision aid, insurance, health, insurance benefits, health priorities

Summary CHAT is a computer-based exercise to engage the lay public in the design of health insurance benefits. The exercise is being converted to a web-based exercise in order to permit larger numbers of individuals to easily participate without all being in the same place simultaneously and without the aid of a facilitator. The first iteration of the web version of the CHAT exercise will be developed by December 2003. A survey firm has been awarded a contract to perform the user evaluation of the website. The study protocol has been reviewed by the Office of Human Subjects Research and exempted from Institutional Review Board review. Recruitment of study subjects will begin at the end of 2003, and the evaluation, which will involve 27 users, will take place in January and February of 2004.

Z01 CL010522-01

Title	Updating Risks and Benefits in Phase I Oncology Trials: A Meta-Analysis
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Christine Grady, PhD (CBD, CC)
Supervisor of Record	Ezekiel Emanuel, MD, PhD (CBD, CC)
Collaborator, Lab	Elizabeth Horstmann (CBD, CC)
Collaborator, NIH	Seiichiro Yamamoto (NCI)
Total Staff Years	.6
Human Research	Human subject research: Interviews
Keywords	phase I oncology, risks, benefits
Summary	This study is a meta-analysis of the responses and toxic death rates of all phase I oncology studies initiated between 1991 and 2002 and sponsored by Cancer Therapy Evaluation Program (CTEP)/National Cancer Institute. The goal is to update our understanding of responses and toxicities in phase I oncology trials. A second goal is to describe the diversity of phase I oncology trials and to show that relying on a single value to understand response or toxicity is misleading.

Z01 CL010523-01

Title Ethical Problems of Registered Nurses and Social Workers

Dates from: 10/01/2003 to: 09/30/2003

Lead Investigator Christine Grady, PhD (CBD, CC)

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

Collaborators, Lab Marion Danis, MD (CBD, CC)
Connie M. Ulrich, RN, PhD (CBD, CC)

Collaborators, NIH Adrienne R. Farrar (SWD, CC)
P. O'Donnell (INOVA)
C. Taylor, PhD (Georgetown University)

Total Staff Years .3

Human Research Human subject research

Keywords ethical conflict, nurses, social workers, job satisfaction

Summary This is a survey of practicing nurses and social workers in four states, representing different regions of the United States. It asks respondents to describe the ethical issues they face in their practices and the amount of conflict they experience, as well as their work environments, ethics resources and support, and job satisfaction. The survey instrument will be sent by mail in the fall of 2003.

Critical Care Medicine Department

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Z01 CL000036-16

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .15

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/mm3; we administered IL-2 for 5 days about every 2 months at doses ranging from 6 to 18 million units/d. The courses of IL-2 were well tolerated, although most patients required dosage reductions due to adverse effects. Sustained improvement in CD4 number was seen primarily in patients with greater than 200 CD4 cells/mm3. There also was a transient increase in viral load as measured by the bDNA assay seen at day 6 to day 8 following initiation of IL-2 therapy. Reductions in CD4 counts 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 combination with currently approved antiretroviral therapies in patients with CD4 counts above 200 cells/mm. 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 because some patients receiving prednisone developed avascular necrosis of the hip. 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 benefit patients with HIV infection.

Z01 CL000037-16

Title Characterization of *Pneumocystis carinii* Surface Antigens

Dates from: 10/01/2002 to: 09/30/2003

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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 used to identify and characterize antigens of this organism. Recombinant antigens can then be used to examine host immune responses to *P. carinii i*nfections. 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 high-performance liquid chromatography (HPLC). It is necessary to use *P. carinii* from both sources because the organisms are different species. 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. We developed an enzyme-linked immunosorbent assay (ELISA) to examine antibody responses to these antigens 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 PCP with the hope that we can use this information to control or prevent this disease.

Z01 CL000073-15

Title Investigations of New Therapies in Septic Shock

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .61

Human Research Neither human cells nor tissues

Keywords septic shock, new therapies

Summary Septic shock and related sequelae of infection (e.g., multiple organ system failure) are the most common cause of death in intensive care units. Deaths due to sepsis can occur in previously healthy individuals, in all age groups, and in a variety of common clinical settings. Those commonly predisposed 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 gallbladder disease. Half of all children or adults who acquire this 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 that places 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). Thus successful treatment of the septic shock syndrome, which reduces 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 successfully provided information on the pathophysiology and treatment of human disease. This model of acute and chronic infection simulates the course and cardiovascular changes seen routinely in children and 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. There are several therapies under investigation that might be effective in human septic shock. The canine model, which simulates the cardiovascular

changes seen in children and adult humans with septic shock, is ideally suited for pre-clinical trials of these new therapies. The canine model allows properly controlled trials to evaluate therapeutic mechanisms and adverse effects of therapies; that 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 vs. norepinephrine vs. the combination as a therapy for shock. Other agents to be tested include AG556 and tyrosine 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.

Z01 CL000146-10

Title Characterization of Immune Responses During *Pneumocystis carinii* Pneumonia

Dates from: 10/01/2002 to: 09/30/2003

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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 (PCR) 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 have begun to conduct gene chip studies 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 PCP and may provide mechanisms for increasing clearance of *P. carinii* or decreasing the inflammation that may be causing hypoxia.

Z01 CL000149-10

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .55

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 that 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 downregulate basal transcriptional activity as measured by mutation and deletion reporter gene constructs. A manuscript has been submitted.

Z01 CL000171-08

Title Inflammatory Responses to Bronchial Endotoxin Instillation in Humans

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1.35

Human Research Human subject research: tissues or cells

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.

Z01 CL000182-08

Title Study of Control of Cytosolic Phospholipase A2 Activity

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .85

Human Research Human subject research: tissues or cells

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 characterized. Recombinant cPLA2 has been produced in bacteria and in insect epithelial cells. Treatment of these proteins with thiol modifiers reduces activity. Treatment with S-nitroso-glutathione also alters activity. Ongoing studies include production of recombinant protein and modulation of enzyme function by oxidant molecules. Two manuscripts are published.

Z01 CL000188-06

Title Endothelial Dysfunction and Vascular Inflammation

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .9

Human Research Human subject research: tissues or cells

Keywords nitric oxide synthases, nitric oxide

Summary

Introduction and Objective: Septic shock marks the point in a severe infection when cascading responses overwhelm compensatory mechanisms, resulting in overt cardiovascular failure. The appearance of vasopressor requiring hypotension substantially increases the risk of death from infection. Up to 75 percent of septic shock non-survivors die in refractory shock during the first 7 to 10 days of illness. In refractory septic shock, both vascular relaxation and constriction ultimately become impaired, an abnormality analogous to endothelial dysfunction and injury in chronic atherosclerosis. This investigation is exploring the mediators, signal transduction pathways, and underlying mechanisms of endothelial dysfunction and vascular inflammation.

Progress: Transfection of monoblastoid U937 cells with human eNOS resulted in a cell line that produces nitric oxide in response to a calcium ionophore, but little or no nitric oxide in the resting state (*Blood,* 1997). However, after differentiation with phorbol-12-acetate-13-myristate, eNOS-expressing cells produced increased amounts of both TNFα and reactive oxygen species by mechanisms that were independent of nitric oxide. Neither N^{Ω}-methyl-L-arginine, a NOS inhibitor, nor mutation of the L-arginine binding site of eNOS, rendering it incapable of producing nitric oxide, blocked the ability of eNOS to upregulate TNFα. Conversely, co-transfection with superoxide dismutase or deletion of the NADPH binding site of eNOS completely prevented eNOS from upregulating TNFα production. These results suggest that eNOS can regulate inflammatory responses through both nitric oxide (*J Immunol*, 1994; *J Biol Chem,* 1997) and reactive oxygen species-based signal transduction pathways (*J Biol Chem,* 2000).

Superoxide produced by eNOS was shown to upregulate TNFα via p42/44 MAPK activation (*J Biol Chem*, 2001). Proposed

Course of Work: Experiments are underway to globally characterize the effects of high mobility group protein (HMGB1), a late endogenous mediator implicated in septic shock mortality, on the transcriptome of primary human endothelial cells. This inflammatory mediator is being combined in a 2x2 design with cycloheximide to separate out secondary regulatory events that require protein synthesis from those that do not. We are exploring of nitric oxide-triggered signal transduction pathways in a human-mouse hybrid endothelial cell line. Initial studies demonstrate that exogenous nitric oxide inhibits proteosome function and activates p38 MAPK. We developed an in vitro model of endothelial cell dysfunction using an RNA-mediated interference (RNAi) approach in primary cells. We are currently constructing plasmids designed to knockdown eNOS expression. Endothelial dysfunction has been associated with reduced eNOS expression or function in a wide variety of models and clinical settings, including sepsis and atherosclerosis. Gene knockdown will be followed by phenotypic characterization using Western blot, flow cytometry, and oligonucleotide microarrays in the presence and absence of inflammatory-mediator activation (TNFα, HMGB1). In parallel experiments, RNAi will be used to knockdown the BMPR2 gene. Loss of BMPR2 function has been linked to primary and secondary pulmonary hypertension, a form of endothelial dysfunction that affects the pulmonary vasculature. At present, we plan to combine BMPR2 knockdown with eNOS knockdown in a 2x2 design followed by phenotypic characterization and expression profiling. Data from this *in vitro* work will be examined and analyzed in the context of clinical samples from a protocol (in preparation with Michael Solomon, MD) enrolling patients with primary pulmonary hypertension.

Z01 CL000189-06

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .9

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 bronchoalveolar lavage have been 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. Oral washes have been collected prospectively from patients at San Francisco General Hospital who have HIV infection and possible pneumocystis pneumonia. Quantitative PCR had a high sensitivity and high specificity for identifying patients with pneumocystis pneumonia, especially if a cut-off of 50 copies/µL were used. This study is completed (Larsen H, *J Infect Dis,* in press). Further studies using improved methods to solubilize the clinical specimen are underway.

Z01 CL000191-06

Title Studies of Lymphocyte Kinetics in Healthy and HIV-Infected Patients

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .3

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 given to HIV-infected patients. BrDU is incorporated into DNA and incorporation can be measured using an anti-BrDU monoclonal antibody. By FACS analysis, both surface markers and BrDU can be measured. Thus, FACS analysis can be used to directly measure subpopulations of cells that have replicated. To date, 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. We have also examined lymphocyte kinetics in patients receiving IL-2 and have found that intermittent IL-2 therapy expands both CD4 and CD8 cells, which then have a very long survival in responding patients. Naive and central memory CD4 cells appear to be preferentially expanded. 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.

Z01 CL000192-06

Title Molecular Studies of Human *Pneumocystis Carinii*

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .8

Human Research Human cells or tissues

Keywords *Pneumocystis carinii*

Summary *Pneumocystis carinii* infections remain common in HIV-infected patients despite the broad use of highly active antiretroviral therapies and prophylactic regimens. Studies of human *P. carinii* are focusing on two areas: diagnosis and evaluation for potential resistance to therapy. To try to develop highly sensitive, 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 family of genes is closely related and encodes 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 used primers. We are currently evaluating these primers prospectively in collaboration with the Microbiology Department and investigators at San Francisco General Hospital and the Center 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 (PCP) 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 PCP. 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, characterized the kinetics of this enzyme, and 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 SSCP (single strand conformational polymorphisms) and have examined a large number of samples for DHPS mutations, including organisms obtained from an Italian cohort. We have also 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.

Z01 CL000198-06

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .69

Human Research Neither human cells nor tissues

Keywords septic shock, tyrphostin AG556

Summary Septic shock appears to result from excessive release of cytokines [e.g., tumor necrosis factor-α (TNF-α), IL-2] 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 are 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 depends 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 depends 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 were not helpful and might be harmful. Therefore, it may be 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 of standard dose 2.5 mg/kg AG 556 to placebo, to titrated dosing 1mg/kg, and then 1 or 4 mg/kg depending on the blood pressure of animals at the 6-hour point. This is the first study in an animal model to examine whether the utility of anti-inflammatory therapy depends on the burden of infectious agent. It has potential implications for human clinical trials of anti-inflammatory agents in sepsis.

Z01 CL001157-05

Title Effects of Inhaled Nitric Oxide on Pulmonary Inflammatory Responses

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .5

Human Research Human subject research: tissues or cells

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 H2O2 production, CD11b/CD18 expression, and less IL-6 and IL-8 in BAL fluid compared to 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. Following the endotoxin instillation, subjects will breathe NO (40 ppm), delivered by an anesthesia non-rebreathing face mask with a reservoir bag; control 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.

Z01 CL001158-05

Title Nitric Oxide Regulation of Inflammatory Responses and Gene Expression

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .95

Human Research Human cells or tissues

Keywords nitric oxide

Summary *Introduction and Objective:* Nitric oxide (NO) is an important inter- 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. Furthermore, inhaled NO is under investigation as a therapy for respiratory distress syndrome in neonates and adults. In addition to its vasodilating effects on the systemic and pulmonary vasculatures, NO also modulates immune responses and regulates gene expression. These latter attributes may have implications for the use of NO synthase inhibitors or NO itself for syndromes in which the host's inflammatory response plays a pathogenic role. In this project, we are examining the role of NO as a modulator of inflammation and gene expression.

Progress: Human phagocytes, in particular neutrophils, lacked the capacity to endogenously produce NO under a number of ex vivo and in vivo conditions (*J Immunol,* 1994). Therefore, the ability of these cells to be regulated in a paracrine manner by exogenous sources of NO such as the endothelium has been explored. In addition to upregulating TNFα production (*J Immunol,* 1994), NO was found to modulate IL-8 mRNA levels and IL-8 production in human neutrophil preparations (*J Infect Dis,* 1998). We have confirmed that endogenously produced NO also upregulates TNFα production using human U937 cells, a monoblastoid cell line, transfected to express murine inducible NO synthase (*Blood,* 1997). Investigation of TNFα regulation by NO resulted in the description of a cGMP-independent signaling pathway that utilizes cAMP downregulation as a signal transduction event (*J Biol Chem,* 1997). A NO-responsive Sp1 binding site was identified in the proximal TNFα promoter (*J Biol Chem,* 1999). NO-mediated decreases in cAMP leads to reduced Sp1 binding to the TNFα promoter with subsequent increases in

TNFα transcription. Recent work has shown that NO downregulates the eNOS promoter through effects on Sp1 identical to those that cause TNFα upregulation (*J Biol Chem,* submitted 2003). For TNFα, an AP1 site upstream to Sp1 serves to reverse the direction of the NO response. Mutation of the AP1 site converts the effect of NO in TNFα from up to down regulation (eNOS-like). The IL-8 promoter lacks a canonical Sp1 site. Unlike TNFα, IL-8 regulation by NO is both cGMP and cAMP-independent. Further, NO p38 MAPK-dependent effect on protein binding to regulation of IL-8 has been found to be post-transcriptional and mediated via AU-rich elements in its mRNA 3' UTR (manuscript in preparation, 2003). An oligonucleotide microarray analysis in differentiated U937 cells has identified more than 100 additional NO-regulated genes (manuscripts in preparation, 2003).

Proposed Course of Work: Current work is focused on identifying all major pathways of NO gene regulation using microarray and molecular biology approaches. Part of this effort is directed toward characterizing NO effects on the cell-cycle and the respective roles of p38 MAPK, proteasome inhibition, and mRNA stability on gene regulation by NO. Separate experiments are directed at profiling the effects of NO and inflammatory mediators such as endotoxin, TNFα, and HMGB1 on a variety of primary cell types including neutrophils, elutriated monocytes, and endothelial cells.

Z01 CL001159-05

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .41

Human Research Human subject research

Keywords MRI, 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 AVN 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 MRIs. The study will elucidate the natural history and risk factors for this unexpected complication of treated HIV disease. This follow-up continues. A formal evaluation will be performed after 3 to 5 years of follow-up to determine the natural history of this disorder.

Z01 CL001160-05

Title Study of the Control of P11 Protein Production

Dates from: 10/01/2002 to: 09/30/2003

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

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Total Staff Years .65

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 retinoic 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. Three manuscripts have been published.

Z01 CL001164-04

Title Functional Genomics of Critical Illness

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1.7

Human Research Human subject research: cells or tissues

Keywords critical illness, genomics

Summary

Introduction and Objective: Microarray technology and proteomic approaches promise to become pivotal tools in understanding the functional genomics of complex diseases. Critically ill or injured patients frequently die of incompletely understood conditions, such as septic shock, acute respiratory distress syndrome, and ultimately multiple organ dysfunction syndrome. Host inflammatory pathways are thought to play major pathogenic roles in these syndromes. At a basic level, the clinical and biological manifestations of host responses are determined or at least reflected by quantitative and qualitative changes in gene expression. Therefore, organ injury syndromes might be defined by their associated patterns of altered gene expression. Oligonucleotide microarrays can measure relative changes in mRNA levels for thousands of genes simultaneously, providing a global snapshot of the transcriptome. The goals of this line of investigation are: 1) to link pathophysiology and/or interventions with responding genes; 2) to identify system characteristics, signaling pathways, and regulatory networks important to critical illness; and 3) to discover biomarkers, prognostic indicators, and potential targets for drug development.

Progress: Laboratory procedures for handling a variety of sample types, including neutrophils, peripheral blood mononuclear cells, T-lymphocytes, whole blood bronchoalveolar lavage, spleen, liver, lung, and heart, have been developed. We have done preliminary testing of amplication procedures and have worked with oligonucleotide microarrays for several species including human, mouse, and rat. We have constructed and done extensive testing of a data analysis pipeline and established a database and Web-based analytical tools. We investigated interactions between interferon g and dexamethasone in primary bronchial epithelial cells and identified the expression profiles associated with sickle cell anemia, endotoxin challenge in normal volunteers, and escalating severity of infection in a rat model.

Proposed Course of Work: We will define the *in vitro* response of primary human cells including neutrophils, monocytes, and endothelial cells to endotoxin and other immune activators. We will determine whether infection with different types of bacteria produces organism-specific signatures in a rat model of pneumonia. Using knockout mice with specific genetic defects, we will serially examine the severity of sepsis-induced myocardial dysfunction, associated changes in gene expression, and outcome. We will investigate the transcriptomic response of whole blood to smallpox vaccination, identify biomarkers of disease severity and outcome in sickle cell anemia, examine the host response to Severe Acute Respiratory Syndrome (SARS) virus in comparison with the “common cold”-causing coronaviruses and other respiratory pathogens.

Z01 CL001165-04

Title Influence of Site, Severity, and Type of Infection on Effects of Endotoxin Analogue in Sepsis

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .15

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 that 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, which often use 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 IV 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 hours and 24 hours combined but decreased lung lavage neutrophils at 4 hours while increasing them at 24 hours. 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 non-specific 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 impacted 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. This project has been completed and a manuscript is in preparation.

Z01 CL001168-04

Title Gene Expression and Protein Profiles in Humans Challenged with Endotoxin

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1.6

Human Research Human subject research: 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 on the administration of endotoxin, a bacterial wall component, to normal volunteers. By administering endotoxin either intravenously or via intrabronchial instillation, we are able to study early inflammatory events that occur in the blood and in the local environment of the lung. Intravenous endotoxin results in a systemic inflammatory response 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 two-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. It will provide a means of characterizing new mediators and mechanisms that are part of the acute phase response to bacterial products.

Z01 CL001169-04

Title Inflammatory Effects of High Mobility Group Protein 1

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .8

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 (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 TNF, 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 its 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.

Z01 CL001170-03

Title	Global Initiative to Characterize Antiretroviral Pharmacokinetics
Dates	from: 10/01/2002 to: 09/30/2003
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Total Staff Years	.27
Human Research	Human subject research
Keywords	HIV, developing world, pharmacokinetics, antiretroviral, generic, genetics, ethnicity, pharmacogenomics

Summary

The overwhelming majority of HIV-infected persons live in the developing world, so recent efforts have focused on providing antiretroviral pharmacotherapy to this population. However, 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 (i.e., P-glycoprotein) as well as generic formulations of antiretroviral medications may also contribute to altered pharmacokinetics among these patients. The purposes of this pilot, hypothesis-generating study are (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 include subjects that 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 liquid chromatography/mass spectrometry. Population pharmacokinetic parameter values (Cmax, Cmin, AUC, spectrometry 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. This human study should begin in the first quarter of 2004. In a first phase of ensuring comparability of products, tablets containing nevirapine (from six international sources representing three manufacturers) were assayed for drug content and all were found to contain the labeled amount of drug (200 mg); these results were presented at two international meetings, as well as in the *Journal of the American Medical Association (JAMA),* May 28, 2003.

Z01 CL001171-03

Title Expression Profiling in Acute Cardiac Allograft Rejection

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 2.2

Human Research Human subject research: 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 are applying functional

genomics to study acute cardiac allograft cellular rejection. Our group has developed and tested standard laboratory procedures for sample processing and, if necessary, amplification. We have established laboratory and bioinformatics infrastructure to support oligonucleotide microarray investigations. Three major local transplant programs (Johns Hopkins University, University of Maryland, and INOVA-Fairfax) have agreed to collaborate. We have an Institutional Review Board-approved protocol and are currently actively enrolling patients (15 patients enrolled to date). 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 are harvested from heart transplant recipients during periods of immunological 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. Other analytic tools that may be employed include proteomics, real-time polymerase chain reaction, Western blot, *in situ* hybridization, immunohistochemistry, and histopathology. 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).

Z01 CL001172-03

Title	Determinants of Cardiac Function in a Canine Model of Septic Shock
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Steven Solomon, PhD (CCM, CC)
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Collaborators, NIH	Andrew E. Arai, MD (LCE, NHLBI) Michael A. Solomon, MD (CCM, CC)
Total Staff Years	.6
Human Research	Neither human cells nor tissues
Keywords	sepsis, MRI, cardiac mechanics, diastolic function

Summary

The potentially reversible myocardial depression of sepsis is well documented in humans and animals by radionuclide scans and intravascular catheter techniques. The mechanism of sepsis-induced myocardial depression remains incompletely understood. Sepsis-induced myocardial dysfunction cannot be explained by inadequate myocardial oxygen supply or insufficient myocardial high-energy synthetic capabilities. Investigators have postulated a myocardial depressant factor of sepsis, but the mechanisms by which bacteria, their toxins, and host cytokines disturb normal cardiac function remain unknown. 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 the depression of LV ejection fraction but not the LV dilatation in humans and animals. 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 non-invasive MRI data in order to develop an approach suitable for future human studies. This study is also designed to definitively determine if sepsis-induced myocardial depression is associated with microvascular flow abnormalities and LV dilatation. Control studies showed good correlation on MRI imaging and invasive measures. Several sepsis studies were performed but the mortality was greater than expected and MRI/hemodynamic procedures could not be performed. The study will be continued after the sepsis model is modified.

Z01 CL001173-03

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1

Human Research Human subject research

Keywords nitric oxide, sickle cell anemia, hemoglobin, blood flow, pulmonary hypertension

Summary Our studies suggest that secondary pulmonary hypertension is common in adult patients with sickle cell disease, appears to be resistant to hydroxyurea therapy, is linked to hemolysis, and is associated with high mortality. The current study evaluates the following: (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 disease. (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. (4) The effects of 3 months of exchange transfusion on pulmonary artery pressures, other hemodynamic parameters, exercise tolerance, and symptoms in patients who do not receive or fail to respond to NO therapy. We have enrolled 26 subjects in the clinical trial with 13 completing stage I, nine completing stage II, and six completing stage III. There have been no adverse events related to the inhalation of nitric oxide, and approximately 80 percent of patients are responding with decreases in pulmonary pressures.

Z01 CL001174-03

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1

Human Research Human subject research

Keywords nitric oxide, sickle cell anemia, hemoglobin, blood flow, pulmonary hypertension, echocardiogram

Summary Sickle cell disease is an autosomal recessive disorder and the most common genetic disease affecting African-Americans. Mortality rates in sickle cell patients with pulmonary hypertension are hypothesized to be significantly higher than in patients without pulmonary hypertension. We have enrolled 200 patients in a study of the prevalence and prognosis of patients with sickle cell disease and pulmonary hypertension. All patients were screened with transthoracic echocardiograms and the tricuspid regurgitant jet velocity (TRV) used to estimate the pulmonary artery systolic pressure. Pulmonary hypertension was prospectively defined by a TRV > = 2.5 m/sec and severe pulmonary hypertension defined by a TRV > = 3.0 m/sec. Right heart catheterization was performed in consenting patients with TRV > = 2.8 m/sec. Based on these data, 32 percent of patients with sickle cell disease have elevated pulmonary artery systolic pressures (TRV > = 2.5 m/sec), and 9 percent have severely elevated pressures. Multiple-regression analysis identified increasing age, increased serum markers of hemolysis (LDH, total bilirubin) and arginine/ornithine ratio as significant independent predictors of pulmonary hypertension. Fetal hemoglobin levels did not predict pulmonary hypertension nor did hydroxyurea therapy modify pulmonary pressures. Left ventricular dysfunction was rarely observed (< 2 percent of patients) and calculated pulmonary artery systolic pressures based on TRV accurately predicted measured values during right heart catheterization (r = 0.98; p = 0.001). The patients diagnosed with pulmonary hypertension had significantly

greater mortality. These studies suggest that secondary pulmonary hypertension is common in adult patients with sickle cell disease, appears to be resistant to hydroxyurea therapy, is linked to hemolysis and is associated with a high mortality. These data suggest that all patients should be screened for this complication and considered for therapeutic trials with oxygen, anticoagulation, transfusion and/or selective pulmonary vasodilators. Patients continue to be enrolled in this trial and referred for treatment studies if identified with pulmonary hypertension.

Z01 CL001178-03

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .3

Human Research Neither human cells nor tissues

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 using 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 that show novel selectivity for superoxide anion itself. Superoxide anion, however, may have different effects during sepsis. Superoxide may play opposing roles in microbial killing and secondary inflammatory tissue injury, and it also 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 depending 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 for challenge 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., < median), M40401 had the opposite effect.

Z01 CL001179-02

Title Differentiation of Acute Rejection from Infection in a Rat Heart Transplant Mode

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1.7

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. We have established an ACUC protocol that allows us to study whether gene microarray analysis of peripheral blood mononuclear cells (PBMC) will reliably differentiate acute heart rejection from infection in the transplanted rat. The ACUC protocol also allows us to do pilot studies necessary to support the main protocol. To date, we have established the surgical techniques necessary to successfully perform and maintain the rat transplant model. We have established a dose of cyclosporin (CSA) in this model that reliably suppresses rejection during its administration, but will permit the emergence of Grade 3 rejection upon its discontinuation. We have also determined the appropriate inocula of intra-bronchial *E. coli* bacteria that is sufficient to cause a pneumonia and a systemic inflammatory response without being immediately lethal in transplanted rats receiving CSA. In addition, we have used gene microarray technology to study the time course of post-surgical inflammatory changes in order to determine the most opportune time to harvest the transplanted hearts (i.e., when gene microarray signatures due to surgical inflammatory changes are dissipating). Currently we are enrolling animals in the main study protocol. Our main protocol combines two well-established rat models: the first is a heterotopic heart transplantation model and the second is an *E. coli* pulmonary infection model. All rats will undergo heart transplantation on day 0 in conjunction with daily CSA (10 mg/kg subcutaneous) to suppress rejection. After transplant, animals will be randomized at day 4 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 on day 13 to receive intrabronchial *E. coli* inoculation or saline inoculation. Consequently, four groups will be studied: No rejection (i.e., receiving CSA) without infection; no rejection (i.e., receiving CSA) with infection; rejection (i.e., not receiving CSA) without infection; and rejection (i.e., not receiving CSA) with infection. On day 14, all animals will be sacrificed and the blood and heart removed for gene microarray analysis. Other analytic tools that may be employed include: real-time polymerase chain reaction, Western blot, *in situ* hybridization, proteomics, immunohistochemistry, and histopathology. In addition, the animals' lungs, spleen, liver, and thymus will also be procured in the primary study and preserved for potential future analysis.

Z01 CL001180-02

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .9

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"). We have an ACUC-approved protocol to perform a controlled, randomized survival study of IABC in a well characterized low cardiac output animal model of sepsis. We have tested various IABP sizes in the animals to determine the levels of diastolic augmentation and systolic assistance that can be provided. The commercially available sizes did not provide adequate augmentation, so a custom-sized IABP was developed and tested. The custom IABP provided excellent augmentation during short-term testing and subsequently underwent long-term testing (48 hours) to establish if it caused any alterations in renal

function or direct injury to the kidneys. The custom IABPs did not alter renal function and did not cause injury to the kidneys. The proposed study is the first controlled, randomized survival study of IABC in a well-characterized low cardiac output animal model of sepsis. There are three phases in this study: phase 1 (baseline), phase 2 (sepsis), and phase 3 (recovery). During the sepsis phase all animals will receive bacterial clot (18 x 109 CFU of *E. coli*) intravenous fluids (Ringer's solution with 5 percent dextrose) and antibiotics (ceftriaxone). In addition, 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 to 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. We have begun the sepsis protocol and have performed three cycles of the experiment to date. 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, thereby decreasing mortality during sepsis.

Z01 CL001181-02

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1.15

Human Research Neither human cells nor tissues

Keywords sepsis, vasopressors, vasopressin, norepinephrine

Summary The purpose of this clinical study is to examine the role 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 using them 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 combining these two drugs will produce less vasoconstriction-related organ injury and an increased 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. We determined in a pilot study that 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. We will for the first time in any model of sepsis determine the effects of vasopressin and norepinephrine on blood pressure, cardiac output, and survival. The results of this study suggest that the combination treatment of vasopressin and norepinephrine was more beneficial but, due to early mortality, further experiments will have to be performed to provide sufficient statistical power.

Z01 CL001182-02

Title	Early Detection of Arrhythmogenic Right Ventricular Cardiomyopathy
Dates	from: 10/01/2002 to: 09/30/2003
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Total Staff Years	.6
Human Research	Human subject research: minors; cells or tissues
Keywords	arrhythmogenic right ventricular cardiomyopathy, 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 limitation of imaging and diagnostic techniques. This 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 is being prepared for submission to the Institutional Review Board.

Z01 CL001183-02

Title Interferon Induction of Gene Expression in Human Lung Epithelial Cells

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .85

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. Human lung epithelial cells were treated with interferon gamma or dexamethasone or both for 8 or 24 hours. Interferon induced an increase in gene expression of over twofold or greater of approximately 300 genes. Interferon treatment decreased expression by 50 percent or more of over 300 genes. Cotreatment with dexamethasone inhibited interferon-induced changes in gene expression of a variety of inflammatory and cell cycle genes. Changes in gene expression were confirmed at the RNA and protein level in 12 genes. A manuscript is in preparation.

Z01 CL001184-02

Title Effect of Epinephrine in a Canine Model of Septic Shock

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .75

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. The current canine model of sepsis uses a bacterial clot and is treated with fluids and antibiotics. The purpose of this study is to establish the number of animals necessary to determine the dose of epinephrine that will maintain blood pressure during sepsis and to determine if the epinephrine 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. The results of this study have shown that epinephrine, compared to norepinephrine and vasopressin, adversely affects survival rate despite titration to produce appropriate effects on MAP. This manuscript is in preparation.

1 Z01 CL001185-02

Title Effect of Sympathetic Blockade in Non-lethal Sepsis

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .5

Human Research Neither human cells nor tissues

Keywords epidural, morphine, bupivacaine, pain, sepsis

Summary We used a well-established canine model of human sepsis to investigate 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 bupivacaine and morphine, and the other 8 received 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 CFU/kg-1 bw). At comparable doses of intraperitoneal implanted *E. coli* (2.5-5 x 10^9 CFU/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. First, to understand the relationship between sympathetic tone and control of bacterial clearance and proinflammatory mediator release. Second, whether pain relief can be modulated to be effective without negatively affecting survival. This paper was published this year.

1 Z01 CL001186-02

Title An Anthrax Lethal Toxin Model of Sepsis

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .3

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 disseminates systemically by intravascular spread, in which causes an increasing toxin release that contributes directly to death. Anthrax bacilli produce two different virulence factors, including a polyglutamate capsule and a three-component exotoxin. The capsule resists phagocytosis while the toxin is capable of injuring and killing the cells that 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. To date, all small animal models 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 to the same weight-based bolus dose. A similar pattern of mortality is observed in Fischer animals infused with toxin. Using this model we have shown that, in contrast to similarly lethal lipopolysaccharide, anthrax lethal toxin shock is not associated with inflammatory cytokine or nitric oxide release. We have also shown that monoclonal antibody to the protective antigen component of lethal toxin is protective in the model administered up to 12 hours after initial exposure to toxin. Ongoing studies are determining the nature of the protective effect of PA-Mab. Plans are also underway to compare gene expression patterns with anthrax lethal toxin and LPS, to study the mechanisms underlying the cardiovascular abnormality associated with lethal toxin, and to study the effects of conventional hemodynamic support with fluids and vasopressors in the model. It is anticipated that studies will be expanded in a mouse model, where the interactions of the lethal and edema factor components of lethal toxin will be tested.

Z01 CL001187-02

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

Dates from: 10/01/2002 to: 09/30/2003

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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 that 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 pro-inflammatory plasma proteolytic cascades, as well as the cellular production of pro-inflammatory molecular mediators (e.g., cytokines, adhesion molecules, growth factors, oxidants, nitric oxide). In addition to the release of pro-inflammatory mediators, this response is associated with the production of anti-inflammatory molecules, which 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. However, such a characterization may be essential for the application of new therapies designed to modulate inflammation during sepsis. Oligonucleotide microarray analysis is a rapidly growing technology that identifies individual or groups of genes that are up- or downregulated 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 a 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), which are either up- or downregulated in dose ordered fashion during graded bacterial infection either acutely, subacutely, or at recovery (i.e., 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 microarrays under study that do show significant dose-ordered expression levels. However, up- or downregulation with infection, which is readily apparent at 6 hours, does not demonstrate dose-ordering until 24 hours. Furthermore, the majority of genes showing this pattern are downregulated, not upregulated. The relevance of these generalized expression patterns are now being analyzed in functionally related groups of genes as well for individual genes that generate products strongly associated with either the harmful or beneficial effects of the inflammatory response.

Z01 CL001188-02

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .31

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 diethyltriaminepent-acetate (DTPA) Iron III. This agent is a low molecular weight scavenger of free NO that does not 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 improved survival rates with either intrabronchial or intravascular *E. coli*, and in most cases survival rates were reduced. Thus, in this rat model of sepsis, DTPA Iron III appeared to have very similar effects to those observed with NOS inhibitors in patients with sepsis. To confirm this, additional experiments are now being conducted that appear thus far to substantiate the NO scavenging capability of DTPA in the model. The effects of these alterations on myocardial function will be tested shortly.

Z01 CL001189-02

Title The Influence of Infection Duration on TNFMAb in Sepsis

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .21

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 study of TNF Ab, however, we had to develop a clinically relevant mouse model of sepsis showing that the addition of fluid and antibiotic treatment would have the beneficial effects 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, either antibiotics 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 allow us to evaluate the effects of varying treatment times with conventional sepsis therapies on the effects of anti-inflammatory agents like TNF Ab.

Z01 CL008048-01

Title Endothelial Dysfunction as a Risk Factor in HIV

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .7

Human Research Human subject research

Summary Premature atherosclerosis is an emerging problem for patients with HIV infection who are treated with antiretroviral drugs. It is not clear whether these complications are related to HIV, the drugs, or the dyslipidemias that the drugs produce. A study has been developed to look at flow-mediated dilation in patients with HIV treated with antiretroviral drugs. This technique is a surrogate for coronary artery disease that is well established and is an assessment of endothelial function. In this study, groups of 20 antiretroviral-treated patients will have their flow mediated dilation measured and then will be started on one of three consecutively enrolled study arms, including prevastatin and rosiglitazone. Patients will be assessed to determine if the intervention improved their flow-mediated response, and whether other markers of endothelial cell function improve. The first arm is fully enrolled and is currently being reviewed by the Data and Safety Monitoring Board.

Z01 CL008050-01

Title Blood Studies of Endothelial Function and Systemic Inflammation

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .17

Human Research Human subject research

Keywords endothelial function, leukocyte gene expression, RNA, DNA

Summary The collection of human blood from both patients and healthy volunteers is necessary for the development of laboratory assays required for studies of the role of nitric oxide, inflammatory mediators, and endothelial function in inflammatory diseases that involve the blood vessels. To date we have enrolled 28 individuals and collected blood for experimental assays numerous times.

Z01 CL008051-01

Title Use of Hydroxyurea and L-arginine or Sildenafil in Patients with Sickle Cell Disease

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 1

Human Research Human subject research

Keywords nitric oxide therapy, fetal hemoglobin induction, L-arginine, sickle cell disease, Sildenafil (Viagra)

Summary Hydroxyurea is a cell-cycle specific agent that blocks DNA synthesis by inhibiting ribonucleotide reductase, the enzyme that converts ribonucleotides to deoxyribonucleotides. Hydroxyurea has been shown to induce the production of fetal hemoglobin (HbF), initially in non-human primates and now in patients with sickle cell anemia. The majority of patients with sickle cell disease respond to the drug with a more than twofold increase in HbF levels; in some patients the percent of HbF exceeds 10 or 15. We have found that hydroxyurea exerts many of its effects via the production of nitric oxide gas. In this study we treated patients chronically with hydroxyurea to determine hematological changes longitudinally. Once a maximal Hb-F-raising effect of hydroxyurea has been established, oral L-arginine (the substrate for NO synthase) and sildenafil (Viagra, a phosphodiesterase inhibitor that potentiates cGMP dependent signaling) is added to determine the ability of other agents to enhance HbF synthesis, especially in hydroxyurea non-responders or partial-responders. We began enrolling patients in May 2003 and have treated 25 patients to date, 14 with hydroxyurea and L-arginine and 11 with hydroxyurea and sildenafil.

Z01 CL008052-01

Title Atorvastatin Therapy to Improve Endothelial Function in Sickle Cell Disease

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Gregory J. Kato (CCM, CC)

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Total Staff Years .61

Human Research Human subject research

Keywords 3-hydroxy-3-methylglutaryl (HMG) co-A reductase, inhibitors, endothelial function, leukocyte gene expression

Summary The statin class of drugs has been shown to reduce stroke and myocardial infarction in patients with hypercholesterolemia. Intriguingly, the statins have been shown to accomplish this not only by reducing cholesterol levels, but also by improving nitric oxide bioactivity and thereby reversing endothelial dysfunction in human subjects. Atorvastatin is one of the most widely prescribed drugs in the United States, with a well-characterized and very acceptable side effect profile. Our group and others have published evidence that about half of patients with sickle cell disease have physiological and biochemical evidence of impaired nitric oxide bioavailability. This appears to contribute to impaired regional blood flow in patients with sickle cell disease, particularly during vaso-occlusive episodes. Therefore, it is attractive to test atorvastatin for its hypothetical ability to restore nitric oxide-dependent

blood flow in patients with sickle cell disease. We will measure forearm blood flow by plethysmography to determine the response to infusion of L-NMMA, a nitric oxide synthase inhibitor, to which sickle cell patients have a blunted response. After 4 weeks of oral outpatient atorvastatin therapy, this study will be repeated, with increased responsiveness to L-NMMA as the primary outcome variable. Atorvastatin-induced alterations in blood flow to acetylcholine and to nitroprusside will also be evaluated. Secondary studies will evaluate the degree to which the elevated level of xanthine oxidase in sickle cell patients inhibits nitric oxide-mediated blood flow; markers of inflammation and oxidation; and gene expression by microarray and pilot studies of proteomics in sickle cell patients. As of October 28, 2003, we have obtained Institutional Review Board approval to begin these studies, and we now await Food and Drug Administration approval of an investigational new drug for the standard drugs infused in study subjects to assess nitric oxide-dependent blood flow. We anticipate beginning enrollment in mid-December 2003. We plan to evaluate up to 25 subjects, with an interim analysis at 15 patients. These physiological translational studies will determine whether additional studies of statins should be performed to evaluate their efficacy in reducing the clinical severity of sickle cell disease.

Z01 CL008053-01

Title Effect of Intra-Aortic Balloon Counter Pulsation in a Canine Model of Septic Shock

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Steven Solomon, PhD (CCM, CC)

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

Collaborators, Lab Katherine J. Deans, MD (CCM, CC)
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Stephen Richmond (CCM, CC)

Total Staff Years .6

Human Research Neither human cells nor tissues

Keywords intra-aortic balloon pump, sepsis, septic shock

Summary The purpose of this study is to examine the role of intra-aortic balloon pump counterpulsation in the treatment of septic shock. In many patients, the hemodynamic compromise characteristic of septic shock is multifactorial. Early in the development of sepsis syndrome, the combined cardiopulmonary and peripheral vasodilatory effects of sepsis result in a reduced ability to maintain blood pressure in the periphery. However, as sepsis progresses, myocardial dysfunction emerges as an additional contributor in circulatory failure. Thirty percent of patients who die from sepsis are noted to have low cardiac output. This myocardial depression is characterized by a reduction in contractility and dilatation of the left ventricle, which impairs the heart's ability to respond to fluid resuscitation, a mainstay in the treatment of septic shock. This myocardial dysfunction, in combined with the peripheral vaso-dilation seen in early sepsis, leads to circulatory collapse, multisystem organ failure, and death. We hypothesize that the intra-aortic balloon pump may reduce the myocardial depression of sepsis by improving coronary blood flow and reducing left ventricular work, resulting in a decrease in mortality during sepsis. All preliminary studies were performed and the technical aspects of the study have been completed. The main study is ongoing with three cycles (of ten cycles) having been completed.

Z01 CL008054-01

Title Effect of Nitric Oxide on End Organ Injury in a Canine Model of Acute Hemolysis

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Steven Solomon, PhD (CCM, CC)

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Stephen Richmond (CCM, CC)

Total Staff Years .7

Human Research Neither human cells nor tissues

Keywords nitric oxide, hemolysis

Summary This study examines the therapeutic value of inhaled nitric oxide (NO) in attenuating the vascular effects and organ damage resulting from intravascular hemolysis in a canine model. Hemolysis, the *in vivo* destruction of red blood cells, causes a flux of cell-free hemoglobin (cell-free hemoglobin or stroma-free hemoglobin) into the circulation. Cell-free hemoglobin, when present in large quantities, scavenges NO, an endogenous vasodilator produced by the vascular endothelium. The balance between the production of NO by the vascular endothelium and scavenging of NO by hemoglobin during hemolysis partially determines NO bioavailability. Rapid NO scavenging by cell-free hemoglobin disrupts this balance. This disruption in NO homeostasis permits unopposed vasoconstriction to occur, which can lead to end organ injury. We have developed NO consumption methodologies that conclusively show that elevated levels of hemoglobin consume NO in vivo. By applying these methodologies to a canine model of hemolysis, we hope to determine whether the administration of inhaled NO will attenuate the systemic effects of hemolysis. This study will test our hypothesis that inhaled NO will bind cell-free hemoglobin and therefore attenuate the end organ damage incurred during acute hemolysis. If this study proves that there is a beneficial effect of NO therapy in acute hemolysis, it will serve as the basis for future human clinical trials. The model development for producing hemolysis has been completed. An appropriate sedation protocol is being developed followed by the main study.

Z01 CL008055-01

Title Influence of a New Anesthetic Regimen on the Canine Model in Septic Shock

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Steven Solomon, PhD (CCM, CC)

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

Total Staff Years .6

Human Research Neither human cells nor tissues

Summary Canine gene microarray technology has recently become available. This technology uses a blood sample to detect genes that are being expressed during the course of an illness. This study will investigate whether differences in patterns of gene expression correlate with the severity of illness during sepsis and differentiate between survivors and non-survivors. The study is designed to examine the genetic mechanisms of sepsis. By using four different bacterial levels (0, 6, 12, 18 x 109 CFUs), we will produce a range of infection severity that should produce distinct patterns of gene expression. This design allows for the detection of trends in both activation and inactivation of the genetic pathways involved in producing septic pathophysiology. In addition, unlike small animal models, the canine sepsis model allows for serial measurements over time and will allow for comparison of genetic changes in survivors versus non-survivors. This information could identify new targets for therapy, new tests that will determine the presence and severity of sepsis, and possibly markers that predict survival.

Z01 CL008057-01

Title NF-kappa B in Murine Sepsis

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .28

Human Research Neither human cells nor tissues

Summary

Despite the use of effective antibiotics in combination with cardiopulmonary support, the mortality rate from sepsis and septic shock for the last decades has remained high (29 percent). Furthermore, the incidence of sepsis and septic shock appear to be increasing. New therapeutic approaches with wide clinical applicability are needed to lower the high mortality rate of this syndrome. Excessive release of inflammatory mediators contributes directly to the pathogenesis of organ injury and death occurring during severe infection complicated by sepsis and septic shock. Nuclear factor kappa B (NF-kB) is a nuclear transcription regulatory protein central to the activation of several different genes encoding proteins associated with the inflammatory response during sepsis. Under normal conditions, NF-B remains sequestered in an inactive state in the cytoplasm under the control of its cytoplasmic inhibitor (IB) proteins. However, differing kinds of stimuli including LPS (the toxic moiety of gram-negative bacteria) and cytokines (e.g., TNF and interleukin-6) cause the phosphorylation, ubiquitinylation, and subsequent degradation of I-B proteins in turn resulting in the activation of NFB. Then the DNA-binding subunits of NF-B migrate into the nucleus and activate expression of target genes that code for proteins in the inflammatory and immune responses, such as chemokines, cytokines, inducible nitric oxide synthase (iNOS), and adhesion molecules. Many of these gene products have been closely associated with the pathogenesis of the hemodynamic instability and organ injury occurring during sepsis and septic shock. Therefore, agents designed to inhibit NF-kB may have broad anti-inflammatory effects that could be beneficial during sepsis. However, many of the host mediators associated with the inflammatory response and under the control of NF-kB also contribute to innate immunity and the clearance of bacterial infection. Suppression of NF-kB during sepsis could therefore also worsen underlying infection. The present protocol is testing the effects of agents designed to modulate NF-B in a murine model of sepsis. The first agent under investigation is parthenolide. Parthenolide is a sesquiterpene lactone derived from Asteraceae plants.

Work has shown that parthenolide specifically inhibits NF-kB activation by preventing degradation of I-kB and I-kB. Parthenolide has been reported to improve survival when administered up to 3 hours following intravenous LPS stimulation in mice or rats challenged with intraperitoneal or intravenous LPS, respectively. However in the investigations that have thus far been completed in a fluid supported mouse model under this protocol, inhibition of NF-kB with parthenolide has been harmful with LPS challenge. These results emphasize the potential protective effect NF-kB has in host defense against microbial toxins. The mechanisms underlying this effect are under investigation.

Z01 CL008058-01

Title West Nile Disease

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Henry Masur, MD (CCM, CC)

Collaborators, Lab Amy Guillet Agrawal (CCM, CC)
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Total Staff Years .95

Human Research Human subject research

Keywords emerging infections, West Nile, multicenter

Summary West Nile disease has developed into a major mosquito-borne health problem in the United States. No therapy is known to be effective. Animal models suggest that immune serum may be beneficial. A national protocol has been developed in collaboration with the National Institute of Allergy and Infectious Diseases Viral Studies Group to study the efficacy of high-titer immunoglobulin, obtained from Israel, compared to appropriate placebos. The study is randomized and double blinded. It has opened at 30 centers nationally, including the NIH Clinical Center. Six patients have been enrolled to date, including one at the Clinical Center. This protocol is a model for intramural-extramural collaboration to meet the challenge of a new, emerging infection.

Z01 CL008059-01

Title Severe Acute Respiratory Syndrome

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Henry Masur, MD (CCM, CC)

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Total Staff Years .85

Human Research Human subject research

Keywords emerging infection, SARS

Summary Severe Respiratory Distress Syndrome (SARS) suddenly emerged as an international threat in 2002-2003. Little is known about its natural history or management. A protocol has been developed and approved to bring patients to the Clinical Center to study the natural history of this disease, with special emphasis on duration of viral shedding and host response to the virus. This protocol is open to enrollment should the disease develop during the 2003-2004 season. A protocol is also in development in collaboration with Chinese researchers to pherese donors who have recovered from SARS in order to produce a high-titer immunoglobulin that could then be used for a therapeutic trial in the United States or China. This is being done in collaboration and consultation with the Chinese health authorities and Food and Drug Administration.

Z01 CL008060-01

Title Preclinical and Clinical Investigations in Septic Shock

Dates from: 10/01/2002 to: 09/30/2003

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Collaborator, NIH John D. Bacher, BS, DVM, MS (SRU, SSB, OD)

Total Staff Years .7

Human Research Human cells or tissues

Summary *Introduction and Objective:* Septic shock is a highly lethal syndrome initiated by severe, overwhelming infection. This condition is the leading cause of death in Intensive Care Units in the United States. The underlying mechanisms of this syndrome remain incompletely understood despite more than a half century of scientific investigation. These studies seek to examine septic shock pathogenesis and to explore the potential of novel therapeutic strategies using both small and large models of the syndrome, patients with sepsis, and normal volunteers challenged with endotoxin.

Progress: Early studies focused on pathophysiology comparing gram positive and gram negative organisms, the role of endotoxemia, and the efficacy of anti-endotoxin therapies such as lipid A analogs and antibodies. Nitric oxide was examined as an important mediator of septic shock. Non-selective nitric oxide synthase inhibitors were sometimes toxic and never beneficial. Normal volunteers challenged with endotoxin were found to release increased amounts of nitric oxide. Although ibuprofen blocked endotoxin-induced increases in nitric oxide production, blood pressure was unaffected, suggesting that other mechanisms compensated to maintain vasodilation. More recent work has found that severity of illness (risk of death) influences the therapeutic efficacy of anti-inflammatory agents in septic shock.

Proposed Course of Work: There is an ongoing comparative survival study of commonly used vasopressors in septic shock, including epinephrine, norepinephrine, and vasopressin. Protocols are being developed for lymphocyte adoptive transfer and intra-aortic balloon pump support in septic shock. We also plan to validate and apply information on biomarkers and pathogenic pathways obtained from functional genomic investigations.

Z01 CL008061-01

Title Empiric Antibiotic Therapy for Patients with Pulmonary Infiltrates

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Naomi P. O'Grady, MD (CCM, CC)

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

Collaborators, Extramural Donald Goldmann, MD (Boston Children's Hospital)
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Total Staff Years .75

Human Research Human subject research

Keywords pulmonary infiltrates, empiric antibiotics, resistance

Summary Patients with pulmonary infiltrates receive more than half of the antibiotics prescribed in the intensive care unit (ICU). Many of these patients receive antibiotics for suspected, but not proven, respiratory infections. Yet many of these patients with abnormal radiographs actually have infiltrates of a noninfectious etiology. Although pneumonia is the most common hospital-acquired infection in ICU patients, it is difficult to diagnose definitively because diagnosis relies heavily on radiographic findings that correlate poorly with clinical pneumonia. However, because of the attributable mortality associated with nosocomial pneumonia, the risk of missing a treatable infection often outweighs the perception of minimal risk antibiotic therapy. Consequently, overuse or inappropriate use of antibiotics for the treatment of suspected pneumonia is widespread in this subset of patients and has resulted in the emergence of antimicrobial-resistant bacteria. This protocol is designed to determine whether short-course empiric antibiotic therapy (3 days of meropenem) for patients with new pulmonary infiltrates reduces the emergence of antimicrobial-resistant organisms compared to a standard course of antibiotic therapy (days of therapy with antibiotics of the primary care team's choosing). In addition, it will determine whether hospital length-of-stay and cost can be reduced without affecting patient morbidity and mortality. This protocol targets patients who have new pulmonary infiltrates, yet are at low risk of having pneumonia, as determined using the Clinical Pulmonary Infection score. The protocol has been written, a budget developed, and the research is fully funded. Final approval from the BAMSG Steering Committee is pending. Ten study sites have been identified, and site surveys are being circulated to assess the sites' ability to enroll patients.

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Z01 CL040002-03

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .7

Human Research Human subject research

Keywords renal artery stenosis, renovascular hypertension, contrast MRI

Summary The purpose of this protocol is to determine whether magnetic resonance angiography (MRA) and captopril magnetic resonance (MR) renography can provide comprehensive evaluation of patients at risk for renovascular hypertension. Although renovascular hypertension (renal artery stenosis causing high blood pressure) is unusual, it is 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 normal volunteers and 16 patients to this protocol. We anticipate continued patient accrual to this protocol over the coming year.

Z01 CL040003-01

Title Computer-Aided Detection for Computed Tomography Colonography

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 2.4

Human Research Human subject research

Keywords colon cancer, computed tomography, computer-aided diagnosis colonoscopy

Summary We will analyze computed tomography colonography (virtual colonoscopy) data using computer-assisted diagnosis methods. These methods attempt to identify and characterize colonic polyps automatically, thereby improving physician accuracy and efficiency. We will compare the results of the computer analyses with the "ground truth" data (conventional colonoscopy, pathologic analysis).

Z01 CL040004-01

Title	Computer-Aided Detection for Radiologic Images
Dates	from: 10/01/2002 to: 09/30/2003
Principal Investigator	Ronald M. Summers, MD, PhD (DDR, CC)
Collaborator, NIH	Derrick Cox, MD (NCI)
Total Staff Years	.1
Human Research	Human subject research: Minors
Keywords	computer-aided detection, melanona, pulmonary embolus, bone metastases, computer tomography
Summary	The purpose of this project is to develop computer-aided diagnosis for a wide variety of radiologic images. Examples include pulmonary embolus detection and detection of subcutaneous melanomas on computed tomography (CT) scans. This project uses existing NIH CT scan images.

Z01 CL040005-06

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

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Richard Chang, MD (DDR, CC)

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

Collaborators, Lab Thomas Shawker, MD (DDR, CC)
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Anthony W. Kam (DDR, CC)

Collaborators, NIH Richard O. Cannon, MD (CB, NHLBI)
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Total Staff Years .75

Human Research Human subject research

Keywords deep vein thrombosis, thrombosis, thrombolytic therapy, recombinant tissue plasminogen activator (rtPA)

Summary The objective of the study is to evaluate thrombolytic therapy using recombinant tissue plasminogen activator (rtPA) for treatment of acute deep vein thrombosis (DVT) of the lower extremity. While the conventional therapy, anticoagulation, is highly effective in preventing life-threatening pulmonary embolism, it does not preserve venous function in the affected leg, often leading to postphlebitic syndromes. 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 the start of the study, 17 patients have been treated. All except one patient had significant improvement. Five patients also had evidence of pulmonary embolism by lung scans prior to start of their treatment. Only two patients have had evidence of small asymptomatic pulmonary emboli that developed during treatment, detected on ventilation perfusion lung scans that were obtained on 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. Over a follow-up period that ranges from 3 months to 5 years, none of our patients have developed either recurrent deep vein thrombosis or progression to postphlebitic syndromes. Pharmacokinetic studies in our patients have made major contributions to improving our understanding of thrombolytic therapy and toward development of safe, affordable, and effective regimens that will preserve the quality of life for victims of DVT.

Z01 CL040006-05

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

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Ahalya Premkumar (DDR, CC)

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

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Diane A. Johnson (DDR, CC)

Collaborators, NIH Allison Baumann (NCI)
Jennifer Eng-Wong, MD (NCI)
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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 are available on the gynecologic effects of raloxifene in premenopausal women. The purpose of our study is to examine both the short- and long-term effects of raloxifene on ovulation frequency, endometrial development, and cyclic function in general. The study started enrolling patients in January 1999. To date, 15 patients have been enrolled. One subject was dropped because her irregular menstrual cycles made her ineligible. Two other subjects dropped because logistics prevented them from complying with multiple study evaluations. As the parent protocol (98-C-0123) has completed accrual, no more patients will be enrolled into this protocol, either. Therefore, the protocol accrual has been completed, but we have not yet answered the research objectives.

Z01 CL040007-05

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

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Peter Choyke, MD (DDR, CC)

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

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

Collaborators, Extramural Thomas Foo, PhD (General Electric Medical Systems)
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Azita Moalemi, MD (Mt. Vernon Cardiology)
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Total Staff Years .6

Human Research Human subject research

Keywords magnetic resonance angiography, atherosclerotic disease

Summary The purpose of this protocol is to discover and develop 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 investigated new methods of imaging these diseased vessels. For example, we are evaluating time resolved (8-second) carotid MRAs using correlation imaging as well as evaluating high-resolution imaging of the calf vessels to improve the resolution of these small vessels. The results are as yet too preliminary but are 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.

Z01 CL040008-05

Title Diagnostic Efficacy of Virtual Bronchoscopy

Dates from: 10/01/2002 to: 09/30/2003

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 .3

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. Virtual bronchoscopy can also be used to guide interventional procedures. The patients studied in this protocol will be those having inflammatory, infectious, or neoplastic pulmonary processes who would have had chest CT for clinical reasons. These patients will be recruited from current NIH protocols. The study design consists of scanning the thorax using thin-section helical CT, followed by 3D surface rendering of the airways and transfer of the digital data to videotape. In one of 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 diagnosing neoplastic chest lesions will be studied. In the fourth part, certain technical problems in the virtual bronchoscopy procedure will be investigated. The patients will have fiberoptic bronchoscopy only for clinically indicated purposes. We anticipate that virtual bronchoscopy will be diagnostically efficacious for disorders that produce a morphologic alteration in bronchial anatomy. There have been no complications. Virtual bronchoscopy has been shown to be useful for detecting stenoses. We now have access to a CT scanner with higher Z-axis resolution and are investigating its efficacy for virtual bronchoscopy.

Z01 CL040009-05

Title	Normal Volunteer Scanning on Magnetic Resonance
Dates	from: 10/01/2002 to: 09/30/2003
Principal Investigator	Peter Choyke, MD (DDR, CC)
Supervisor of Record	King C. Li, MD (DDR, CC)
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Collaborator, NIH	D. Thomasson, PhD (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 that 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. Over the past year, the accomplishments of this protocol include optimizing imaging on the new 3 Tesla MRI unit, developing new methods of performing MR angiograms of the runoff vessels of the lower extremity, improving the performance of prostate MRI particularly in improving MR spectroscopy, motion tracking for knee and patella movement, functional MRI of the brain, evaluating oxygenation in the brain, and stroke protocols. We have made substantial gains in technical development in all of these areas and there have been no complications. This protocol, therefore, provides an invaluable resource to the NIH by allowing optimization of MRI in normal volunteers in order to apply these improvements to clinical patients.

Laboratory of Diagnostic Radiology Research

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Z01 CL008038-04

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

Dates from: 10/01/2002 to: 09/30/2003

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

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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 given clinical researchers an 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 (CBF) will be increased by inhalation of carbogen (an air mixture containing 6 percent CO_2 and used to calibrate experiments for determining oxygen consumption). To date, 12 subjects (seven males and five females) received the air mixture containing 5 percent CO_2, and nine subjects (five males and four females) received the air mixture containing 6 percent CO_2. Cerebral blood flow increased by 20+/-8 percent due to inhaling 5 percent CO_2, and by 36 +/- 7 percent due to inhaling 6 percent CO_2. Within-session reproducibility was tested by comparing the relative increases in CBF from the two CO_2 intervals within each session. Averaged over all subjects, the difference between the relative CBF increases in the first and second CO_2 intervals was not statistically significant ($p < 0.05$). The standard deviation of the difference was 8 percent of the mean relative CBF increase. These results served as a basis for the submission of a new protocol to study the effect of 6 percent CO_2 in patients with multiple sclerosis to determine if changes in CBF would be similar in patients compared with age and gender-matched controls.

Z01 CL090001-11

Title Magnetic Resonance Imaging in Multiple Sclerosis

Dates from: 10/01/2002 to: 09/30/2003

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

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Collaborators, NIH Henry F. McFarland, MD (NIB, NINDS)
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Total Staff Years 1.65

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 (Zenapex) 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 both clinical and MRI-measured MS disease activity in patients who were previously considered nonresponders to conventional therapy. Studies are underway to see if Zenapex alone can be used as front-line therapy in MS. A phase 2 trial is underway 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. Enrollment has started of active early relapsing remitting MS patients into this study, and changes in enhancing lesions compared with baseline disease activity will be used as primary outcome measure. T1-black holes (BHs) on MRI represent areas of edema, axonal loss, or astrogliosis and correlate with disability in MS patients with. We investigated the heterogeneity of BH appearance over 48 consecutive months in relapsing remitting MS patients, the role of contrast enhancing lesions (CEL) on BH formation, and the role of CEL duration on BH duration over time, and observed that formation of BHs is related to the amount of CEL. However, once those lesions have been formed, the duration of BHs is most likely a consequence of the duration of the enhancement and blood-brain barrier disruption. This new observation on persistent BH over time indicated that it is possible that axonal loss and gliotic scars can resolve on MRI about 21 months after their initial appearance, coinciding with an acute inflammatory lesion. Recently, new vessel proliferation and formation (angiogenesis) has been observed in autopsy and biopsy specimens from patients with MS. In the newly formed MS lesions, there was a striking

increase in the number of new vessels, and similar patterns were observed in and around older lesions, areas of remyelination (i.e., new myelin formation on axons, shadow plaques), and normal-appearing brain tissue. Experimental and clinical studies are being performed to quantify regional cerebral blood flow (CBF) by comparing CBF of MS patients with age and gender-matched healthy controls using perfusion MRI techniques. In addition, patients will undergo a 6 percent CO2 (hypercapnia) inhalation challenge to determine if vascular responsiveness in MS patients is similar to healthy controls. Comparing the CBF response with hypercapnia will determine if the newly formed vasculature includes properly functioning blood vessels. Normal cerebral blood vessels are exquisitely sensitive to the CO2 in arterial blood, and an increase in CO2 causes CBF to increase through dilatation or relaxation of the muscles surrounding the blood vessels. In the future, changes in the hypercapnia-induced CBF response between MS patients and controls may help to monitor treatment of the disease.

Z01 CL090003-09

Title Functional/Metabolic Imaging in the Brain

Dates from: 10/01/2002 to: 09/30/2003

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Collaborators, NIH Alan Charles McLaughlin, PhD (SCS, NIMH)
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Total Staff Years .2

Human Research Human subject research

Keywords imaging of the brain, magnetic resonance imaging

Summary Functional and metabolic magnetic resonance imaging (MRI) techniques have been evolving rapidly and have tremendous potential for clinical brain disorders research. Clinical activation functional MRI studies are performed at 1.5 and 3.0 Tesla using blood oxygenation level dependent (BOLD) contrast and arterial spin tagging (AST) techniques. Reproducible alterations to cerebral blood flow (CBF) were observed in healthy controls receiving intravenous infusions of a cyclo-oxygenase inhibitor (COX) 1, indomethacin with almost complete suppression of the alteration of CBF to 6 percent CO2 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 an insufficient dose or poor absorption of the medication. We observed that while indomethacin reduced the CBF increase during sensorimotor activation paradigms, it did not significantly affect oxygen consumption (CMRO2) in the motor cortex of the brain during activation. The ratio of the activation-induced CBF increase in the presence and absence of indomethacin was 0.54 +/- 0.08 ($p < 0.001$), while the ratio of the CMRO2 increase in the presence and absence of the drug was 1.02 +/- 0.08 (p = not significant). These results suggest that by combining BOLD/AST studies, one can evaluate the effect of drugs on cerebral oxygen consumption during activation.

Z01 CL090004-10

Title Development and Evaluation of Magnetic Resonance Contrast Agents

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 2

Human Research Neither human cells nor tissues

Keywords magnetic resonance contrast agents

Summary Combining commonly used transfection agents (TA) that have high net positive electrostatic charges with macromolecular high-generation (G) dendrimers (G = 5, 7, 9, 10) conjugated to DOTA and gadolinium (III) ion or superparamagnetic dextran coated iron oxide nanoparticle (SPIO), effectively alters the NMR relaxation properties of these magnetic resonance imaging (MRI) contrast agents. The physical chemical properties of the TA-contrast agents were modeled, 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. Various concentrations of ferumoxides (SPIO) -poly-l-lysine (FE-PLL) complexes were used to label cells magnetically. Rapidly growing cell suspension and adherent cells were labeled effectively via endocytosis into endosomes at low concentrations of ferumoxides (25 micrograms/ml media) and PLL (0.75 micrograms/ml media). Hematopoietic stem cells and lymphocytes required higher concentrations of PLL (1.5 micrograms/ml) in serum-free media during initial FE-PLL complex formation prior to labeling the cells in culture. Total iron concentration in cells depended on the cell type, concentration of FE-PLL complexes in media, cellular density, and incubation time. Iron concentrations following overnight incubation with given ferumoxides at 25 micrograms /ml media resulted in, for example, T cells being labeled with 1–3 pg/cell of intracytoplasmic endosomal iron and 15–20 pg/cell of intracytoplasmic iron in mesenchymal stem cells, compared with 0.01 to 0.1 pg/cell for unlabeled

cells. No adverse effect on the cell viability and functional capacity or toxicity was observed following magnetic cell labeling with FE-PLL. Biodistribution studies of magnetically labeled human mesenchymal stem cells in rats demonstrated that labeled cells could be detected using a 1.5 Tesla clinical magnetic resonance (MR) unit in the liver for up to 29 days after an intravenous infusion of 900,000 cells. FE-PLL-labeled encephalotigenic T cells were injected into recipient mice to induce experimental allergic encephalomyelitis (EAE), a mouse model of multiple sclerosis. FE-PLL-labeled T cells were detected in the spinal cords of EAE mice at the time of initial neurological event using MR microscopy at 7 Tesla. Immmunohistochemical analysis revealed that magnetically labeled T cells had proliferation assays and cytokine profiles similar to those of unlabeled encephalotigenic T cells. In addition, there was an excellent correlation between MR microscopy and histology of spinal cords in clinically affected animals. Plans are to translate the magnetic labeling technique using a Food and Drug Administration-approved MRI contrast agent ferumoxides complexed to PLL to label autologous peripheral blood mononuclear cells or stem cells in order to monitor the temporal spatial of these cells for repair, replacement, or therapy of central nervous system disease.

Z01 CL090005-08

Title Magnetic Resonance Imaging in Experimental Allergic Encephalomyelitis and Remyelination

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 3.15

Human Research Neither human cells nor tissues

Keywords magnetic resonance imaging, allergic encephalomyelitis, remyelination

Summary Magnetic resonance imaging (MRI) scans were performed in the Lewis rat experimental allergic encephalomyelitis (EAE) model, in which magnetically labeled mouse embryonic stem cells (ESC) were implanted into lateral ventricles of the brain. Magnetic resonance (MR) imaging performed at clinically relevant magnetic field strength demonstrated extensive migration of magnetically labeled grafted ESC along the ventricles and into the corpus callosum of these animals. 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* MR microscopy at 7 Tesla. This is the first demonstration of successfully 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. Studies performed using magnetically labeled lymph-node-derived cells in the marmoset EAE model have shown that labeled cells in the brain and spinal cord can be detected on a clinical MRI scanner. These results will serve as the basis for magnetically labeling cells harvested during apheresis and re-infusing autologous cells into patients with multiple sclerosis (MS) to monitor by MRI the migration of labeled stem cells or peripheral blood mononuclear cells into the central nervous system. Serial MRI studies performed in the marmoset EAE model are used for preclinical evaluation of macrophage inhibitory molecule (MIM) for MS. Macrophages are an important component in MS and EAE lesion development, and inhibition of the migration of macrophages into the brain may have an important role in down-regulating the inflammatory processes. Preliminary results comparing MIM with placebo in the marmoset EAE model using clinical, pathologic, and MRI evaluations as outcome measures revealed a possible advantage in the treated animals versus placebo-treated animals in severity of disease. Future studies will use the marmoset EAE model as the basis for harvesting stem cells and transplantation to determine if these cells will stimulate remyelination in EAE lesions.

Z01 CL090006-11

Title Multimodality Radiological Image Processing System

Dates from: 10/01/2002 to: 09/30/2003

Lead 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) has been migrated over to the Clinical Center's new PACS (Picture Archiving and Communication System). 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 PET 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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Z01 CL000401-15

Title Imaging Organ Function in Small Animals

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Michael Green (DNM, CC)

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Total Staff Years 2.9

Human Research Neither human cells nor tissues

Keywords small animal positron emission tomography, small animal single photon emission computed tomography, small animal radionuclide imaging

Summary A commercial annular scintillation camera was purchased and a multiple pinhole collimator array fabricated to create a single photon emission computed tomography (SPECT) scanner for mice. The eight-hole collimator array will be rotated mechanically inside the annular scintillation crystal while projecting eight magnified images of the mouse onto the crystal. This scheme will allow an eight-fold improvement in sensitivity compared with a single pinhole and will allow complete projection sets to be acquired in a very short time. This system is being assembled mechanically and the calibration and image reconstruction software are being written. An advanced signal-processing package was designed to acquire and process data from the Hamamatsu flat panel position-sensitive photomultiplier tube (PSPMT). This tube offers a number of advantages over competing PSPMTs in small-animal positron emission tomography (PET) applications but is subject to the degrading effects of pulse pile-up at high rates due to its large field-of-view (48 mm x 48 mm). Each analog/digital acquisition package, one for each tube, is designed specifically to minimize this effect while retaining the advantages of a large field-of-view. Several of the integrated circuitboards for these packages have been fabricated and tested, and work on the digital elements of the package is underway. During this reporting period, the Imaging Physics Laboratory also supported the work of multiple intramural research groups in PET imaging studies of tumor hypoxia in mice, gene expression in mice, cerebral stimulation studies in the rat, quantitative methodological validation studies in the rat and mouse, and a variety of other biodistribution imaging experiments. Modifications and changes to the software of the ATLAS small-animal scanner continued to be made in response to requests from these investigators and in an effort to enhance the functional capabilities of the system.

Z01 CL000416-05

Title Image Analysis for Quantitative Assessment of Tumor Response to Therapy

Dates from: 10/01/2002 to: 09/30/2003

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Collaborators, NIH Steven K. Libutti, MD (SMS, NCI)
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Total Staff Years .9

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 tomography (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 monitoring 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 therapy in progress, potentially improving both tumor response and patient morbidity. Several areas of investigation are being pursued toward achieving this goal. Some of the principal ones follow:

1) Assessment of the physiologic models used 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 with similar data obtained from Gd-DTPA dynamic MR images. The variability and reproducibility of each of the methods also is 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 fluorodeoxyglucose (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 accepted for publication in the *European Journal of Nuclear Medicine* and 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 a Society of Nuclear Medicine meeting.

3) The above methods, combined with partial volume corrections from CT and factor analysis/principal components analysis, will be used to make objective assessments of the various physiologic parameters (e.g., FDG "uptake"). ROC analysis will be used to determine which of these quantitative indices are 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. A new simplified Patlak Analysis method was developed. Initial results were presented orally at the June 2003 Society of Nuclear Medicine meeting, and a manuscript is currently in review with the *Journal of Nuclear Medicine*. In addition, a manuscript was published in the *Journal of Molecular Imaging and Biology,* describing the blood flow and metabolism measurements as applied to prostate cancer.

1 Z01 CL000417-10

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

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Jorge A. Carrasquillo, MD (DNM, CC)

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Collaborator, Extramural Don Axworthy, PhD (NeoRx)

Total Staff Years 5.5

Human Research Human subject research

Keywords radiolabeled monoclonal antibody imaging, tumors, positron emission tomography

Summary These antibody studies are designed to develop improved methods for detecting and treating malignancies. Our group performs preclinical evaluation of radiolabeled antibodies that appear to be promising after initial screening. We also perform the clinical trials evaluating their pharmacokinetics and dosimetry. Two collaborative radioimmunotherapy trials with Dr. Waldmann (PI), in which we used humanized anti-Tac monoclonal antibody, are ongoing. In order increase the radioactive dose delivered to tumors and improve the tumor-to-nontumor ratios, we are pursuing a pretargeting approach where the antibody with streptavidin is delivered without any radioactivity, and after it targets the tumor, a radiolabeled biotin is injected. This allows the small radiolabeled biotin to localize in the tumor rapidly or be excreted rapidly. The two antibodies we have focused on are B3 and anti-mesothelin (developed in Dr. Pastan's lab). These pretargeting studies have evaluated Y-90 and Bi-213. Dr. Paik has focused his chemistry work on the chemical modification of spherical polymers (PAMAM dendrimers) as carriers of radionuclides and biological molecules. In addition, Dr. Paik has developed a simple purification method for Y-86 that is produced by the NIH positron emission tomography (PET) department.

This Y-86 is to be used for dosimetry of antibodies radiolabeled with Y-90 that are being used by our group. Various protocols using [F-18] FDG in PET and [O-15] water for tumor detection, follow-up, and blood flow measurements are ongoing with NCI's Surgery Branch (Dr. Libutti). There are several ongoing collaborative studies evaluating fluoro-deoxyglucose (FDG)-PET for assessing tumor response to treatment (Dr. Swain, Dr. Mackall, Dr. Sausville, and Dr. Wilson). We have evaluated the use of FDG-PET in assessing sites of viral replication in patients with HIV and have noted interesting changes in nodal visualization in patients with AIDS (Dr. Brust). We are using FDG-PET in attempts to localize sites of activated lymphocytes. In one study, we used FDG-PET to evaluate sites of metabolic activity in systemic lupus erythematosus patients with active versus inactive disease. We also are evaluating the ability of FDG-PET to differentiate between patients with benign versus lymphomatous adenopathy in the setting of autoimmune lymphoproliferative syndrome.

Z01 CL025001-02

Title Optimization of Parameters for Tumor-Targeting of Radiobiologicals

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 2.1

Human Research Neither human cells nor tissues

Keywords radiologicals, tumor targeting, dendrimer-based radiopharmaceuticals

Summary The overall purpose of our research was to improve the tumor-targeting properties of radiolabeled biologicals by optimizing chemical parameters. This year we developed a semiautomated three-column system to purify a positron emitter,Y-86, because it is a better surrogate radionuclide than the In-111 currently in use to estimate dosimetry of Y-90-labeled therapeutic radiopharmaceuticals. This purification system involved the passage of Cyclotron-produced Y-86 samples through a Sr-selective Sr-Spec column, a Y-selective RE-Spec column, and, finally, a cation-exchange Aminex A5 column connected in series. This method enabled us to decontaminate Sr by 250,000 times with greater than 80 percent recovery of Y-86. We also investigated a new Tc-99m chemistry involving [Tc-99m (OH)3(CO)3]+ with Tc-99m (I) oxidation state to label biological molecules under mild conditions. The three water molecules of this complex are labile and can be substituted by a molecule with two or three coordination sites to form a stable ellipsoidal Tc-99m complex. We optimized the chemical condition to label norbiotinamido-DTPA and -EDTA with Tc-99m at 45°C. The animal study indicates that this approach is promising to synthesize a polar Tc-99m-labeled biotin that rapidly accumulates in tumors with pretargeted antibody-streptavidin while being excreted via the renal system.

Z01 CL060001-08

Title	Gene-Specific Radiotherapy
Dates	from: 10/01/2002 to: 09/30/2003
Principal Investigator	Ronald D. Neumann, MD (DNM, CC)
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Collaborators, NIH	Olga Sedelnikova, PhD (NCI) 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 to develop 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 subnanometer 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 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 also have completed development and characterization of a proposed in vitro DSB repair assay using 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 also have 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 also 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 125-I-TFO-induced DSBs to be 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. It also may allow molecular analysis of repair processing at highly complex DSB structures. Such studies are not currently possible due to a lack of knowledge about the actual structure of a complex radiation-induced DSB and which aspects of its structure are biologically important.

POSITRON EMISSION TOMOGRAPHY DEPARTMENT

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Z01 CL000500-07

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

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years 5

Human Research Human subject research

Keywords radiopharmaceuticals, positron emission tomography

Summary Knockout mice have proven invaluable in validating new radiotracers as ligands for a specific receptor. The biodistribution of the proposed binding site-specific radioligand is determined in groups of wild-type and knockout mice homozygous (-/-) for the target of interest. The difference in binding of the radioligand in wild-type and knockout mice can be attributed to the absence of the specific binding site, all other variables being equal. This is important given that doses of drug to inhibit binding at the binding site of interest are rarely specific, especially at the doses needed to saturate the binding site. Therefore, knockout mice represent a solution to the time-consuming process of validating new radiotracers that is quantitative and requires minimal experimentation. The early detection of Alzheimer's disease using positron emission tomography (PET) imaging has thus far not been possible, although several radiotracers are being evaluated. The M1 and M2 receptor subtypes might be suitable targets for investigating Alzheimer's disease, although the autopsy studies were small in number and carried out on patients who had been treated with various drugs. Agonists measure both the receptor density and the affinity state of the receptor, whereas antagonists measure only receptor density. Therefore, agonists are likely to be more useful radiotracers (e.g., the M2 receptor agonist [18F]FPTZTP). Based on experiments involving the radiolabeling and validating of [18F]FPTZTP and [18F]paclitaxel (FPAC), the knockout mouse appears to be the most expeditious method for radiotracer validation.

The pharmacological approach is time-consuming and might not give a definitive answer. A smaller number of experiments are involved in radiotracer validation using knockout mice compared with validating a new tracer by testing saturability, specificity, and distribution characteristics. The knockout mouse approach is therefore more suitable for drug development. Knockout mice represent a clearly defined biochemical change, whereas pharmacological intervention rarely represents a simple biochemical change. Many binding site-specific molecules are not specific at the doses needed to block the binding site in order to prove saturable binding. Regional brain localization of [18F]FPTZTP in M2 receptor knockout mice compared with wild-type mice, M1 receptor knockout mice, M3 receptor knockout mice, and M4 receptor knockout mice clearly shows the preference of [18F]FPTZTP for the M2 receptor subtype. With the availability of knockout mice, these validation experiments can be completed in a matter of weeks rather than the months necessary for the full pharmacological approach. Knockout mice have been used to show that [18F] fluorodeoxyglucose (FDG) is a sensitive probe of changes in 6-glucose phosphatase (G6Pase) levels. The monitoring of gene therapy of glycogen storage disease type 1a in a mouse model was achieved using [18F]FDG and a dedicated animal scanner. The G6Pase knockout mice were compared with knockout mice infused with a recombinant adenovirus containing the murine gene encoding G6Pase (Ad-mG6Pase). Serial images of the same mouse before and after therapy were obtained and compared with wild-type mice of the same strain to determine the uptake and retention of [18F]FDG in the liver. Image data were acquired from heart, blood, and liver 20 minutes after injection of [18F]FDG. The knockout mice retained more [18F]FDG than the wild-type mice.. The mice treated with adenovirus-mediated gene therapy showed [18F]FDG retention similar to that found in age-matched wild-type mice. These studies show that FDG can be used to monitor G6Pase concentration and, therefore, the progress of glycogen storage disease. [18F]FPAC is a sensitive probe for P-glycoprotein (P-gp), a protein responsible for multidrug resistance. Paclitaxel (Taxol) is a clinically important chemotherapeutic agent. [18F]FPAC shows high uptake into and rapid clearance from tissues in rats. Preadministration of paclitaxel in rats significantly increases the retention of [18F]FPAC in blood (33.0 percent increase), heart (32.0 percent), lung (37.6 percent), and kidney (142.4 percent). Biodistribution and radiation dose estimates for [18F]FPAC have been obtained in monkeys, and the effects of a P-gp blocker, XR9576 (Xenova, *http://www.xenova.co.uk)*, on FPAC kinetics also have been studied. Liver uptake of FPAC was affected significantly by XR9576. Studies with mdr1a/1b (-/-) knockout mice showed significant increases in the uptake of [18F]FPAC in the heart, lung, femur, muscle, and brain compared with wild-type mice. Changes in the uptake of [18F]FPAC resulting from preinjection of unlabeled paclitaxel were significant only in the lung and kidney of wild-type mice. Therefore, [18F]FPAC is a substrate for P-gp and might be useful

for *in vivo* imaging of P-gp-mediated efflux. To apply the information obtained from knockout mice to clinical studies, experiments must be carried out to confirm that the PK and PD are similar. Metabolic differences are the most likely confounding factor. With the availability of mouse and human hepatocytes and the improved sensitivity of liquid chromatography/mass spectometry (LC/MS), metabolite identification in both species can be ascertained easily. The use of LC/MS and hepatocyte preparations allows the differences in metabolism between species to be assessed. In humans, the major use of PET has been, and will continue to be, in occupancy studies, either at a single time point or as a function of time after drug dosing. Driven by many advances in technology and the use of knockout mice and LC/MS, PET imaging is rapidly becoming a major force in drug discovery.

Laboratory Medicine Department

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Z01 CL010010-28

Title Magnesium Metabolism in Humans and Biological Systems

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Nadja Rehak (CCS, CC)

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Total Staff Years .4

Human Research Human subject research

Keywords magnesium metabolism

Summary Oral calcium (Ca) supplements (Tums) to plateletpheresis donors is widely practiced to counteract symptoms associated with acute decreases in serum ionized calcium (iCa) concentration due to infusion of citrate anticoagulant. However, the clinical usefulness of this practice has not been evaluated. We studied the associations of donor symptoms and various laboratory parameters in a randomized placebo-controlled study of oral Ca carbonate administration. Twenty-three donors (12 males and 11 females) underwent four plateletpheresis procedures each, receiving in random order elemental Ca (1 or 2 g orally) or a corresponding placebo, 30 minutes before the procedure. Ten of these donors underwent a fifth procedure with a 4g Ca supplementation. All procedures were performed at fixed citrate infusion rates of 1.5 mg/kg/min. Oral Ca induced dose-sensitive changes in parathyroid hormone (iPTH), total calcium (tCa), and iCa. Compared with placebo, the most marked overall attenuation in iPTH release and the most rapid early improvement in tCa and iCa concentrations occurred after the 1g Ca dose. These effects were smallest after the 4g Ca dose. The serum tCa and iCa concentrations one day after the procedure were higher, and iPTH were lower, in the Ca-supplemented group. The urine excretion of Ca and magnesium (Mg) was increased at the end of procedure, was not affected by Ca dose, and returned to baseline within 24 hours of the procedure. Plateletpheresis also induced significant changes in serum alkaline phosphatase, 1,25-dihydroxyvitamin D, and osteocalcin. The severity of donor symptoms during apheresis was associated with the extent of decrease in serum iCa and ionized magnesium (iMg) concentrations, and also with albumin, creatinine, and vitamin D concentrations. Females experienced more symptoms than males. However, the association of gender with symptoms was not significant after adjustment for lower serum albumin, creatinine, and total Mg in the females. Compared with placebo, ingestion of 2g Ca resulted in reduced paresthesias. However, in general, ingestion of Ca did not significantly improve the overall symptom scores. Thus, oral Ca supplementation administered prior to the apheresis procedure resulted in modest improvements in donor symptoms and in critical serum analytes. Two manuscripts are being published.

Z01 CL010241-10

Title Identification of Molecular Defects in Patients with Von Willebrand

Dates from: 10/01/2002 to: 09/30/2003

Lead 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 of factor VIII. 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.

Z01 CL010247-09

Title	Molecular and Phenotypic Methods for Identifying Mycobacteria and Nocardiae
Dates	from: 10/01/2002 to: 09/30/2003
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Collaborators, Extramural	June M. Brown (CDC, BS) Karen C. Carroll, MD (The Johns Hopkins Hospital) Joann Cloud, MS (ARUP Institute) Arnold G. Steigerwalt, BS (CDC)
Total Staff Years	1.1
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 portions 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. Identification of many isolates to the species level can be obtained within a few days of organism isolation using this technique, compared with the month or more required for conventional identification based on biochemical testing. In addition, these molecular procedures allow better discrimination among species and subspecies than is possible with biochemical testing and facilitate the detection of hitherto undescribed species. 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 several such unrecognized Nocardia species; work is ongoing to characterize these organisms further. In addition, we have found several clinical isolates belonging to the species *Nocardia veterana*, newly recognized both as a species and as a human pathogen. A manuscript has just been published that describes our findings on this organism, including the problems with distinguishing it from another newly described pathogenic species, *Nocardia africana*. Some isolates of Nocardia species cannot be precisely identified even with our RFLP procedure or with complete 16S rDNA sequencing. DNA-DNA hybridization is being used to define these isolates more precisely. Characterization of several such isolates,

based both on phenotypic and molecular biologic features, is underway. We also hope to characterize further several interesting Nocardia isolates we have found that appear to possess several different 16S rRNA gene copies per cell. It would be of taxonomic, biochemical, and medical interest to determine the extent of similarity among these genes and to learn which of them is functional. In addition, we have begun collaborations with others who have extensive organism collections to assess the extent to which some of their isolates may belong to species that can be reliably identified only by using molecular methods.

Z01 CL010265-08

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

Dates from: 10/01/2002 to: 09/30/2003

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

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

Collaborators, NIH John E. Bennett, MD (CM, NIAID)
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Leslie B. Calhoun (MICRO, CC)

Total Staff Years .05

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 and allow institution of antiviral therapy at an earlier point in the prodromal stage of CMV disease. We have developed a quantitative 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 signal with this 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. During fiscal year 2003, analysis of the prospective study data has revealed that the real-time PCR assay has a high degree of sensitivity for detecting viremic episodes, which are detected by CMV antigen and a negative predictive of greater than 95 percent for CMV antigen-negative specimens. Publication of the study results is expected in the last quarter of 2003.

Z01 CL010283-07

Title Platelet-Associated Antibodies in Patients with Autoimmune Thrombocytopenic Purpura

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Margaret E. Rick, MD (HEME, CC)

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

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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, but the demonstration of specific antibodies has been difficult for a variety of reasons. In general, when the antibodies can be demonstrated, there is an inverse correlation with the platelet count in individual patients. We have set up an assay for specific platelet glycoproteins to aid in the diagnosis, treatment, and monitoring of patients with ITP. We are using the tests particularly for the follow-up of patients before and after treatment in two studies: 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, and in an ITP treatment protocol for less severely affected patients using daclizumab. Sixteen patients have been studied in the transplant protocol and four have been studied in the daclizumab protocol. A publication regarding the transplant patients and an abstract has been submitted to the American Society of Hematology (December 2003 meeting) regarding the patients with daclizumab.

Z01 CL010287-07

Title Assessment of Lymphocytes in Patients with Autoimmune Lymphoproliferative Syndrome

Dates from: 10/01/2002 to: 09/30/2003

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

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

Total Staff Years .35

Human Research Human subject research: cells or tissues

Keywords autoimmune lymphoproliferative syndrome

Summary An extensive flow cytometric evaluation continues of patients with autoimmune lymphoproliferative syndrome (ALPS) and their extended family members, on the basis of characterization of the expanded double-negative T-cell and B-cell populations. Double-negative T cells have been demonstrated to be alpha beta TcR, CD57+, HLA-DR+, and CD45RA+. This study has been extended to characterize the double-negative T cells more completely, including B220 expression and gamma-delta TcR T cells in all ALPS patients. In addition, we have initiated expanded characterization of the B cells, directed at memory B cells using CD27 and B220 assessment in these patients. 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 if the immunophenotypic findings represent a functional defect in immunoregulatory T cells that could explain the genotype phenotype disparity in families with ALPS type 1a. This approach has met with unanticipated problems in developing a consistent *ex vivo* indicator system for inhibition. It does appear that the assay system is now set and the studies of immunoregulatory T cells in ALPS should be completed in the next fiscal year. In addition, this assay system will be extended to ATL cells as a possible leukemic expansion of the immunoregulatory T cell.

Z01 CL010300-05

Title Identification of Proteolytic Activity for von Willebrand Factor

Dates from: 10/01/2002 to: 09/30/2003

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

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Collaborators, Extramural Stephan Moll (University of North Carolina School of Medicine)
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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 studied a group of 50 masked plasmapheresis samples in collaboration with hematology investigators from the University of North Carolina School of Medicine and shown correlation with clinical status and with another assay for the protease. We are collaborating with Dr. Anita Aggarwal to assess the response to plasma exchange and Dr. William Stetler-Stevenson (NCI) to attempt to express the protein in mammalian cells.

Z01 CL010303-05

Title Development of New Assays for Lipoprotein Testing

Dates from: 10/01/2002 to: 09/30/2003

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

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

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

Collaborator, NIH Glen Hortin (DLM, CC)

Total Staff Years .5

Human Research Neither human cells nor tissues

Keywords lipoprotein testing

Summary Lipoprotein fraction analysis is useful for estimating the risk for coronary artery disease and for assessing the effectiveness of cholesterol-lowering therapy. The objective of this study is to improve the analytical performance of current lipoprotein assays and, potentially, to develop new lipoprotein tests with improved diagnostic accuracy. In the past year, we have developed several alternative detergent solubilization procedures for adapting our previously developed sequential lipoprotein test to other lipoprotein homogenous assay tests that are in routine clinical use. The sequential lipoprotein test makes it possible to measure, in a single reaction tube with an automated instrument, all the major serum lipid and lipoprotein fractions (high-density lipoprotein [HDL]-cholesterol, total cholesterol, triglyceride, and calculated low-density lipoprotein [LDL]-cholesterol), thus significantly simplifying the laboratory procedure for lipoprotein fraction analysis. We also investigated the use of an immunoassay capture technique for the characterization of apolipoproteins, the major proteins on lipoprotein particles, by matrix-assisted laser desorption/ionization time of flight (MALDI-ToF) mass spectrometry. Using this technique, we were able to detect and characterize from serum the molecular forms for apolipoprotein A-I and apolipoprotein E, which are possible diagnostic markers. For example, some molecular forms of apolipoprotein E have been shown to be associated with the development of Alzheimer's disease, but the currently used tests for identifying the different molecular phenotypes of apolipoprotein E are very labor-intensive and expensive to perform. In the future, we plan to develop an assay for performing apolipoprotein E phenotyping by immunoassay capture followed by MALDI-ToF tandem mass spectrometry, which will allow the detection and differentiation of the various forms of apolipoprotein E by their molecular weight. We also plan to investigate several precipitation and filtration methods for developing a test that can be performed by routine clinical laboratories for measuring prebeta HDL, a small HDL particle that recently has been shown to be a better negative predictor for coronary artery disease than large HDL particles.

Z01 CL010304-05

Title	Mutation Analysis of Selected Lymphoid Immune Disorders
Dates	from: 10/01/2002 to: 09/30/2003
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	.45
Human Research	Human subject research: 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] (autoimmune lymphoproliferativsyndrome) (ALPS) are being evaluated using direct gene sequencing with fluorescent probes. These studies have continued to identify a number of new mutations in both diseases, and these data have been either 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. This procedure, and the manuals and technical approaches used, are being used to assist with the NIH CLIACLIA (Clinical Laboratory Improvement Amendment of 1988) resource program in areas of molecular diagnostics. The project also has provided valuable teaching opportunities for fellows in training. This facility is being linked into the Medical Genetics training program in the National Human Genome Research Institute.

Z01 CL010305-05

Title Analytical Performance and Clinical Utility of Thyroid Function Tests

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Gyorgy Csako, MD (CCS, CC)

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Total Staff Years .2

Human Research Human subject research

Keywords thyroid function tests, thyroid cancer, lipids, lipoproteins, hemostasis, atherothrombosis, lipoprotein(a)

Summary Both hypothyroid and hyperthyroid patients have been reported to experience an increased incidence of atherothrombotic events. We completed analysis of thyroid function tests, serum lipid parameters (with special attention to lipoprotein[a]), and various hemostatic parameters in previously thyroid-ectomized patients who underwent thyroid scan for follow-up of their thyroid cancer. At that time, the patients' thyroid status changed from hyperthyroid (due to thyroid hormone-suppression therapy) to temporarily hypothyroid (due to discontinuation of thyroid hormone-suppression therapy). The blood specimens obtained during these phases represent primarily systemic thyroid hormone effects as opposed to underlying diseases with confounding effects of inflammation and so forth found in primary hypothyroidism and hyperthyroidism. Since all previous studies compared different groups of hypothyroid and hyperthyroid patients, the results of our studies with the same patients in different thyroid status are expected to provide a clearer understanding of the effect of thyroid hormones on components of atherothrombosis.

Z01 CL010306-05

Title Analytical Performance/Clinical Utility of Laboratory Tests for Atherothrombosis

Dates from: 10/01/2002 to: 09/30/2003

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Total Staff Years .85

Human Research Human subject research

Keywords atherosclerosis, thrombosis, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), lipoprotein(a), infection, inflammation, lipopolysaccharides, C-reactive protein

Summary We examined serum markers for atherothrombotic risk in several studies: (1) Electrophoresis coupled with enzymatic detection of cholesterol in agarose gel has the advantage of allowing simultaneous measurement of cholesterol in all major lipoprotein fractions at a comparatively low cost. Although these methods have been evaluated analytically, information on possible interferences is limited. We observed a case in which gross errors occurred in the measurement of LDL-C and VLDL-C with such a commercial method. Based on our detailed analysis of the patient's serum lipoproteins, the erroneous LDL-C and VLDL-C results were due to a fast-migrating, abnormally composed LDL that changed over a 6-month period. However, occurrence of this anomalous LDL could not be correlated with medications, other laboratory findings, and/or clinical course. While the frequency of this

anomalous LDL among clinical specimens and with other cholesterol electrophoretic methods is not known, this case suggests that unexpected and/or unexplainable combination of very low LDL-C and high VLDL-C results with these methods should be verified by alternative techniques. (2) In a collaborative study, we studied patients with sickle cell disease for endothelial nitric oxide bioavailability. Similar to atherosclerotic vascular disease, sickle cell disease is characterized by chronic inflammation and ischemia-reperfusion injury. Plasma C-reactive protein levels were indeed elevated in these patients and were correlated with other markers of inflammation, such as ferritin and white blood cell count. Further, we found that endothelial nitric oxide was more bioavailable in females than in males. The findings may explain the sex differences reported for morbidity and mortality in these patients. (3) In a collaborative study, we observed that, probably through multiple effects on a cell response to stress, high levels of human heat shock protein 70 are associated with low coronary artery disease risk. (4) In another collaborative study, we showed that Cla-1, the human orthologue of rodent scavenger receptor BI, not only is a major receptor for HDL that mediates reverse cholesterol transport, but also is able to bind and internalize both monomerized, lipoprotein-free and HDL-associated bacterial endotoxins (lipopolysaccharides). This suggests that Cla-1 may play an important role in septic shock by affecting the cellular uptake and clearance of lipopolysaccharides.

Z01 CL010307-05

Title Development and Clinical Application of Molecular Diagnostic Tests

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Gyorgy Csako, MD (CCS, CC)

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

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Collaborator, NIH Trey Sunderland, MD (NIMH)

Total Staff Years .6

Human Research Human subject research: cells or tissues

Keywords polymerase chain reaction, restriction fragment-length polymorphism, single-strand conformation polymorphism, DNA, homocysteine, Alzheimer's disease, systemic lupus erythematosus, thrombosis

Summary An increasing number of genes have been linked to Alzheimer's disease over the past decade. Most recently, hyperhomocysteinemia and gene mutations suspected to cause hyperhomocysteinemia have been suggested as possible contributors to Alzheimer's disease. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is the major circulating form of folate and a cofactor in remethylation of homocysteine. Two different mutations, one at base position 677 (C to T transition that results in a "thermolabile" variant of the MTHFR enzyme) and another at base pair 1298 (A to C transition), are now suspected to lead to increased homocysteine levels and increased risk of Alzheimer's disease. In order to study the possible role of these gene mutations in Alzheimer's disease, we developed practicable polymerase chain reaction (PCR)-single-strand conformation polymorphism methods for their study in a large patient population. Both methods have been validated against conventional PCR-restriction fragment-length polymorphism assays and confirmed by DNA sequencing. In order to analyze the effect of both MTHFR gene mutations on plasma homocysteine, we also are measuring the plasma homocysteine and cysteine levels by high-performance liquid chromatography in these patients. In another collaborative study, the possible pathogenic role of apolipoprotein(a) isoforms and alleles is being studied in a large cohort of patients with systemic lupus erythematosus. These patients often experience atherothrombotic events, and finding reliable predictors for these events is of major clinical importance. Our preliminary findings indicate that apolipoprotein isoforms/alleles may be such predictors.

Z01 CL010308-04

Title Assessment of Memory B Cells in Immune Disorders

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, Lab Margaret R. Brown, MA (IMMUNE, CC)

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

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

Total Staff Years .15

Human Research Human subject research

Keywords memory B cells, immune disorders

Summary A project to develop the complete immunophenotype of memory B cell has been initiated. This is being done by evaluating normal subjects and patients with specific immune disorders, including autoimmune lymphoproliferative syndrome (ALPS) and chronic granulomatous disease (CGD). In addition, the immunophenotypic data are being compared with single B cell Ig gene somatic hypermutation results generated in Dr. Peter 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 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 patient's B cells and the over-expression of certain families among the diminished memory B cells in ALPS, suggesting B-cell repertoire skewing that may be associated with the autoimmunity seen in this disorder. These studies suggest that the Fas pathway may be critical in the generation of memory B cells, while defective NADPH oxidase activity does not impact memory B cell development but does diminish CD27 expression. These studies also point out the CD27 is not a consistently reliable marker of memory B cells in humans.

Z01 CL010316-03

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Daniel Fedorko, PhD (MICRO, CC)

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

Collaborators, Lab Nancy A. Nelson (MICRO, CC)
Esther C. Williams (MICRO, CC)

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

Total Staff Years .35

Human Research Human cells or tissues

Keywords *Clostridium difficile*, diagnosis, polymerase chain reaction

Summary We are evaluating the performance of a polymerase chain reaction (PCR) assay designed in our laboratory for the diagnosis of *Clostridium difficile* disease. This assay uses a primer set to detect the toxin A gene and another set to detect the toxin B gene. Endpoint detection of PCR products is an enzyme-linked immunosorbent assay (ELISA) format. Our PCR assay will be compared with the following methods for diagnosis of *C. difficile* disease: toxigenic culture, cytotoxin assay, and an ELISA for detection of toxins A and B. DNA has been extracted from 443 stool specimens and the PCR assay has been performed on 292 of these specimens so far. Data from the other three assays is available for all 443 specimens. Once all PCR assays have been performed, a chart review will be performed for specimens with conflicting results. Our results will demonstrate if PCR is a useful tool for diagnosis of *C. difficile* gastrointestinal disease.

Z01 CL010317-03

Title Interaction of Plasminogen with Human Platelets

Dates from: 10/01/2002 to: 09/30/2003

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

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Paula K. Merryman (HEME, CC)

Total Staff Years .9

Human Research Human cells or tissues

Keywords plasminogen, platelets, fibrinolysis

Summary Dissolution of blood clots (fibrinolysis) requires plasmin, a protease derived from the activation of plasminogen by tissue plasminogen activator (tPA). Both plasminogen and tPA are known to bind to the surface of platelets, where their interaction becomes greatly accelerated. Therefore, platelets are important promoters of fibrinolysis. Our laboratory has been examining plasminogen binding to platelets in some detail. We use classical equilibrium binding experiments with the goal of establishing the number of binding sites and the binding affinity. We also are chemically crosslinking 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 the platelet membrane protein IIIa and possibly to IIb. When platelets are activated with thrombin, the number of their plasminogen binding sites appears to decrease from approximately 120,000 per cell to 60,000 per cell, but the binding affinity increases several fold.

Z01 CL010319-03

Title Platelet Function in Patients Treated with Selective Serotonin Reuptake Inhibitors Versus Non-Selective Serotonin Reuptake Inhibitors Antidepressants

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigato) Margaret E. Rick, MD (HEME, CC)

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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 subject research: cells or tissues

Keywords selective serotonin reuptake inhibitor antidepressants, platelet function

Summary Selective serotonin reuptake inhibitors (SSRIs) are widely used antidepressant agents, which are known to decrease platelet serotonin content. They have been reported to be associated with bleeding in a minority of patients and recently have been associated with an increase in gastrointestinal bleeding. The purpose of this study is to better understand the potential risks of bleeding associated with mild platelet dysfunction in patients using SSRIs and to determine whether a global test of platelet function, as performed on the platelet function analyzer-100, is able to identify the changes in platelet function associated with SSRI use. We have almost completed accrual on this project and have begun data analysis.

Z01 CL010324-02

Title Evaluation of Hypercoagulability in Patients with Major Depression

Dates from: 10/01/2002 to: 09/30/2003

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); (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 diurnal variation of factor VIII appears to be blunted in depressed patients, with factor VIII remaining higher throughout the day. Approximately one-quarter of the depressed individuals have significantly elevated PAI-1 both in the morning and evening. Insulin infusion increases factor VIII and PAI-1, but the elevations are greater in depressed patients than in control subjects.

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

Z01 CL010325-02

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Daniel Fedorko, PhD (MICRO, CC)

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Collaborators, Lab Pattarachai Kiratisin, MD, PhD (MICRO, CC)
Nancy A. Nelson (MICRO, CC)
Esther C. Williams (MICRO, CC)

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

Total Staff Years .12

Human Research Human cells or tissues

Keywords *Clostridium difficile*

Summary We evaluated the performance of a commercial polymerase chain reaction kit for the detection of *Clostridium difficile* toxin A and B genes directly in patient stool specimens. Results will be compared with data from these traditional tests for diagnosis of *C. difficile* gastrointestinal disease: toxigenic culture, cytotoxin assay, and enzyme-linked immunosorbent assay (ELISA) for toxins A and B. We tested 144 stool specimens by all methods. We are waiting for data from the ELISA tests, which were performed at Hennepin County Medical Center and for results from chart reviews for specimens that gave conflicting results. Once we have these data, the manuscript can be completed and submitted for publication.

Z01 CL010326-02

Title Identification of *Streptococcus Mitis* Species by Housekeeping Gene Sequencing

Dates from: 10/01/2002 to: 09/30/2003

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

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

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

Total Staff Years .3

Human Research Neither human cells nor tissues

Keywords *Streptococcus mitis*, group gene sequencing, bacterial identification

Summary The viridans streptococci are a group of gram-positive bacteria that constitute part of the resident flora in the human oral cavity and gastro-intestinal tract. They occasionally can 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 of 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 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 introduced recently. However, this technique has not been helpful in some closely related organisms that have nearly identical sequences in their 16S genes. The 16S rDNA sequencing approach for identification of clinically important bacteria, however, has real limitations. We decided to try the multilocus gene sequencing targets—housekeeping genes—for differentiating bacteria at the species level, even though this molecular version of starch gel protein electrophoresis has not been widely used for species differentiation. We set out to try this approach for species delineation of bacterial isolates belonging to the mitis and sanguinis groups of viridans streptococci. Although the finer discriminations required for successful strain typing would suggest that this approach might give cleaner distinctions among organisms at the species level, it was unknown whether conserved oligonuceotides for polymerase chain reaction amplification of targeted gene regions could be found for all the species of these two groups, or whether any sequence differences

detected in housekeeping genes in these unexamined species would reliably differentiate organisms at the species level or prove to be just random mutations that are stably present for short evolutionary time periods and, thereby, only useful at distinguishing bacteria strains within a single species. We were able to demonstrate that two of the housekeeping gene targets used for *S. pneumoniae* strain typing also could be used for very clear species discriminations in these two clinically important groups of viridans streptococci. Our studies successfully demonstrated that two of the genes studied, gdh and gki, could be applied to species identification in these organisms. The discriminatory power of this approach is far better than 16S rDNA sequencing. This work has been described in a submitted manuscript.

Z01 CL010327-02

Title Evaluation of Microscopic Stains for Spore-Forming Bacteria

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborators, Lab Daniel Fedorko, PhD (MICRO, CC)
Alexandra T. Wong (MICRO, CC)

Total Staff Years .05

Human Research Neither human cells nor tissues

Keywords spore stain, malachite green, acridine orange, auramine O, *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. We previously compared two staining methods: a hot malachite green stain (requires heating the slide) and a cold malachite green stain. Representative isolates from the spore-forming genera *Bacillus, Paenibacillus,* and *Clostridium* were grown overnight in culture, and then slides were prepared for staining. We found that the hot malachite green stain was superior to the cold stain; however, concern with aerosol formation during the hot staining procedure led us to explore alternative cold stains. We evaluated two fluorescent stains: auramine O and acridine orange. Both fluorescent stains were superior to either the hot or cold malachite green stains. The contrast between spores and vegetative cells (i.e., bacterial cells without spores) was excellent, and the microscopic slide could be examined rapidly using low-power magnification. Because acridine orange stains can be purchased commercially, we found using this dye more convenient than auramine O. The results of this work have been submitted for publication.

Z01 CL010328-02

Title Microbial Identification Using Surface-Enhanced Laser Description/Ionization

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, Lab Frida Stock, BS (MICRO, CC)

Total Staff Years .1

Human Research Neither human cells nor tissues

Keywords matrix-assisted laser desorption/ionization, bacterial identification, proteomics

Summary Bacteria have traditionally been identified 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 and sometimes days for a definitive answer. More recently the use of genomics, such as sequencing ribosomal RNA genes or housekeeping genes, has proved helpful. 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 last 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) and surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-ToF MS). Last year we explored using SELDI for identification of selected gram-positive and gram-negative bacteria. This year, with the multidepartment Clinical Center purchase of the MALDI-ToF MS system, we have expanded our studies to use this system. Preliminary studies indicate that unique, reproducible profiles could be obtained for *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Enterococcus faecalis*. The advantage of the MALDI system is that whole cells could be analyzed, reducing the variability of bacterial lysis, which was required with the SELDI system. The difficulty with the MALDI system is the complexity of the profiles. We believe this can be resolved with the recent purchase of software for data analysis.

Z01 CL010330-02

Title Validation of Rapid Sterility Test Method for Cellular Therapy Products

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, Lab Frida Stock, BS (MICRO, CC)

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Total Staff Years .95

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 United States Pharmacopeia's (USP) "Sterility Test" on all samples containing antibiotics, including cells grown in antibiotic-containing media. The methods for sterility testing described in the Code of Federal Regulations (CFR) and USP standards were developed more than 25 years ago and are labor-intensive and require incubation for 14 days. Since these methods were published, more sensitive and rapid methods have been developed for detection of microbial growth in various body fluids. These modern methods include automated systems for the detection of microbial growth in blood and other normally sterile fluids. Despite the fact 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. Six different cell products from the Department of Transfusion Medicine (DTM) were seeded with selected bacteria (eight strains) and fungi (two strains) (ten colony-forming units and 50 CFUs) and then tested in triplicate with each method. The test sensitivity and time to detect a positive culture were assessed. With each bacterium and fungus, the automated systems were found to be more rapid and sensitive for detection of contaminated cell products. Growth of strict aerobic and anaerobic organisms grew

preferentially in aerobic and anaerobic blood culture broths, respectively; therefore, each cell product would have to be cultured in the two broth formulations. A second but related study was performed for the National Cancer Institute (NCI) to assess use of the automated culture systems for determining sterility of their cell therapy products. The design of the studies was the same as described above although a more limited number of organisms were evaluated with a single cell therapy product. The results of these studies were identical to that reported above. The data collected in these studies have been submitted to the FDA for review. The FDA already has accepted use of the automated culture systems for processing the NCI cell products, and we anticipate the same decision will be made for the DTM cell therapy products. This will result in a more sensitive and rapid sterility testing method and a significant reduction in cost for processing these products. The results of these studies have been presented at the national transfusion medicine meeting, and a manuscript reporting these findings is in preparation.

Z01 CL010331-02

Title Evaluation of Real-Time Polymerase Chain Reaction Assay for Diagnosis of Pneumonia Using Oral Washes

Dates from: 10/01/2002 to: 09/30/2003

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

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Collaborators, NIH Joseph A. Kovacs, MD (CCM, CC)
Henry Masur, MD (CCM, CC)

Collaborators, Extramural Laurence Huang, MD (University of California–San Francisco)
Hans Larsen, MD (Hvidovre Hospital)

Total Staff Years .7

Human Research Human subject research

Keywords Pneumocystis pneumonia diagnosis, oral washes

Summary *Pneumocystis jiroveci* (*Pneumocystis carinii*) is an important cause of 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 valuable 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 blinded, prospective study has been conducted with collaborators at University of California–San Francisco (UCSF) to further evaluate the performance of the real-time PCR assay in detecting PCP. For samples obtained within 1 day of the initiation of PCP therapy, the sensitivity of oral wash samples with QTD-PCR for detecting PCP was greater than 90 percent. The results of this study are in

press. 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. Recent work on an improved method for sample processing has demonstrated that use of DTT markedly improves the homogeneity of the processed oral wash material. If, as expected, this results in more reproducible quantitative results, the positive and negative predictive values for PCP associated with certain levels of signal should improve considerably. We are initiating testing of a series of samples with the processing modifications to test this hypothesis.

Z01 CL010332-02

Title Quantitation of Residual Tumor in Patients Undergoing Allo-Transplantation

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Roger Kurlander, MD (HEME, CC)

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

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

Collaborators, NIH Austin John Barrett, MD (HB, NHLBI)
Alan S. Wayne, MD (POB, NCI)

Total Staff Years .28

Human Research Human cells or tissues

Keywords minimal residual disease, BCR-ABL, polymerase chain reaction, chronic myelogenous leukemia

Summary For 5 years, the molecular diagnostic laboratory in Department of Laboratory Medicine/Hematology has been using a qualitative, nested end-point polymerase chain reaction (PCR) assay to monitor patients with chronic myelogenous leukemia (CML) for residual disease. During the past year, exploiting newer real-time PCR methods, the laboratory has begun collecting information using a quantitative BCR-ABL assay (Q-PCR). This assay measures mRNA levels of the tumor-specific fusion protein BCR-ABL expressed relative to the level of mRNA expression of G6PD, a convenient housekeeper gene. In analyzing results from 102 samples assayed using both the standard and quantitative assays, several features are clear. First, the quantitative measurement of expression levels provides useful additional information about sample integrity not available by endpoint analysis of a housekeeper on an agarose gel. Although all 102 mRNA samples were intact by conventional agarose gel criteria, using Q-PCR to assess housekeeper levels, we found 16 of the 102 samples contained less than 10 percent of the concentration of mRNA expected in optimally treated controls. In almost all cases, these reductions were seen in mail-in samples sent to the laboratory from some distance away. Seven of these samples were positive for BCR-ABL despite poor sample integrity, but the negative result observed in the remaining nine samples must be interpreted with caution. Based on these findings, we are seeking alternative approaches to improve sample integrity. Of particular interest is a planned study to test the value of newly released collection tubes containing RNAse inhibitors. A central

concern in using Q-PCR in the post-transplant setting is its sensitivity. In our experience, comparing conventional nested and Q-PCR assays, 88 of 102 results have been concordant (64 negative and 24 positive samples). We note ten samples positive by the nested assay and negative by Q-PCR, and four samples positive by Q-PCR but not by nested methods. This suggests the quantitative assay may be slightly less sensitive than the nested method. On the other hand, Q-PCR provides valuable insights unavailable using the qualitative nested method. Physicians caring for CML patients post-transplant within the National Heart, Lung, and Blood Institute currently begin treatment for recurrent disease (using donor lymphocyte transfusions or STI when patients more than 6 to 12 months post-transplant display two consecutive positive nested BCR-ABL assay results. Q-PCR reveals the heterogeneity of this population group. Some patients have extremely low-level stable disease at the time of treatment, while others have 100- to 1000-fold higher levels of tumor cells and/or progressive disease. The clinical implications of recurrence in these setting may be quite different. While DLI clearly is indicated for the latter group, watchful waiting for evidence of progression might also be a valid strategy for the former. Q-PCR also permits better monitoring of early trends in therapy. Working with clinical investigators, we hope to be able to better monitor persistent disease and how their interventions affect the clinical course.

Z01 CL010333-02

Title Monitoring Donor T Cell Alloreactivity During Hematopoietic Transplantation

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Roger Kurlander, MD (HEME, CC)

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

Collaborators, Lab Elizabeth S. Chao (HEME, CC)
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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 During the past year, in collaboration with Dr. Richard Childs of the National Heart, Lung, and Blood Institute, we have begun studying T-cell alloreactivity in MHC-matched donor-recipient pairs before stem cell allotransplantation and in recipients post-transplant. To this end, we have adapted ELISPOT methods to enumerate the number of alloreactive cells and to characterize their pattern of cytokine production. Initially, considerable time was spent improving the laboratory methods for cryopreservation and thawing samples, and in developing criteria for evaluating the quality of thawed products. One major concern in comparing immune T-cell responses with donor and recipient stimulator cells is the marked heterogeneity in composition and viability of thawed mixed mononuclear cell preparations. To address this problem, we have adapted published methods using IL-4 and CD40L, which permit the *in vitro* generation of large numbers of homogenous activated B cells from small numbers of mixed mononuclear cells. In comparing the function of these activated B cells with mixed mononuclear cells as APC for stimulating alloreactivity, we find that activated B cells are at least as effective as mixed mononuclear cells in eliciting alloreactive T-cell proliferation. The nature of the alloimmune response, however, is quite different. Mixed mononuclear cells typically evoke effectors with a strong Th1/Tc1 pattern of cytokine production. By contrast, B cell APC powerfully polarize responding T cells in a Th2/Tc2 pattern of cytokine release. We are extending these studies by comparing the impact of B cell and mixed mononuclear cell APC on T-cell responses against viral and ultimately against tumor-related antigen epitopes. Since these patterns of cytokine

production would be expected to have significant functional consequences, these studies have important implications for the use of prepared B cells as APC in a range of other ex vivo immunologic applications. In addition to these differences in cytokine polarization, B and mixed mononuclear cell APC also appear to elicit quantitatively different patterns of T-cell response, perhaps reflecting differences in minor transplantation antigen expression. We are still in the process of analyzing the results of our initial studies using these differing populations of APC to study allo-reactivity before and after transplantation in Dr. Child's patients. We hope in the near future to determine whether the methods we are developing can be used to predict or monitor graft versus host disease or engraftment problems post-transplant.

Z01 CL010334-02

Title Quantitating Engraftment of Donor Hematopoietic Cells After Transplantation

Dates from: 10/01/2002 to: 09/30/2003

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Collaborators, NIH Austin John Barrett, MD (HB, NHLBI)
Michael R. Bishop (ETIB, NCI)
Richard W. Childs, MD (HB, NHLBI)
Douglas Hale, MD (TAB, NIDDK)
Alan S. Wayne, MD (POB, NCI)

Total Staff Years .5

Human Research Human cells or tissues

Keywords donor chimerism, polymerase chain reaction, allotransplantation

Summary The Hematology Molecular Diagnostics Laboratory routinely measures donor T cell and myeloid chimerism in patients undergoing stem cell allotransplantation using a variety of investigator-specific preparative regimens. Based on our accumulated experience we now can recognize several discrete patterns of donor engraftment: (1) Patients undergoing nonmyeloablative conditioning and T-cell-rich stem cell infusions undergo rapid donor engraftment but more delayed myeloid engraftment. In an analysis done with Dr. Childs and his associates (in press), we could identify a number of factors that influence the speed of engraftment in this setting. These include the underlying diagnosis, the level of prior chemotherapy, and the level of concurrent immunosuppression post-transplant. (2) Patients undergoing myeloablative conditioning with T-cell-depleted stem cells demonstrate the opposite pattern; that is, rapid myeloid engraftment with much later donor T-cell engraftment. (3) Patients undergoing myeloablative conditioning with T-cell-replete grafts engraft both cell types quickly. Because of these profound protocol-specific differences, the criteria for successful engraftment vary depending upon the details of each transplantation protocol. To help us interpret this heterogeneous engraftment data, we monitor engraftment data for each protocol group separately and notify individual investigators on an ongoing basis of patients with atypical patterns of engraftment. In recent monitoring

of chimerism patterns, we identified, early in their clinical course, two individuals who ultimately required emergent infusion of additional stem cells and/or T cells for graft salvage, and several other patients with very slow or incomplete engraftment. Poor engraftment in transplant patients could reflect suboptimal stem cell or T-cell dose, unrecognized technical problems during pheresis and cell freezing, or underlying subtle immune abnormalities in the donor or recipient. In the coming year, we will be working with the cell processing unit in Department of Transfusion Medicine focusing on engraftment problems early in the post-transplant period; that is, days 15 through 60 post-transplant. By evaluating in detail the properties of cells administered and, in some cases, by evaluating retrospectively the function of aliquots of the donor cells that were infused using the ELISPOT methods under development in this laboratory, we hope to identify factors underlying instances of poor engraftment and perhaps develop a strategy for avoiding them in the future.

Z01 CL010335-01

Title Heparin Versus Lepirudin in Preventing Withdrawal Occlusion of Venous Access Device

Dates from: 10/01/2002 to: 09/30/2003

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

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Collaborators, NIH Karim Anton Calis, PharmD, MPH (PHARM, CC)
Richard W. Childs, MD (HB, NHLBI)
C. Kasten-Sportes, MD (NCI)

Total Staff Years .2

Human Research Human subject research

Keywords catheter, withdrawal occlusion, lepirudin, heparin

Summary Central venous catheters are essential for the care of patients requiring chronic venous access, yet clots often develop at the catheter tip and block blood withdrawal. Although heparin flushes are routinely used to prevent this, there is little evidence that they are any more effective than saline. This study compares the effectiveness of standard heparin with flushes of a new anticoagulant, lepirudin, given at least daily for the first 3 to 4 weeks after the catheter has been inserted. The measured parameter is the frequency with which the catheters develop withdrawal occlusion.

Z01 CL010336-01

Title Adsorption of Lepirudin to Silicone

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborators, Lab Kimberly Brokaw (HEME, CC)
Ann M. Cullinane (HEME, CC)

Total Staff Years .3

Human Research Neither human cells nor tissues

Keywords lepirudin, silicone, catheters

Summary Central venous catheters typically stimulate intravascular coagulation, which occasionally progresses to occlusive deep vein thrombosis. In an attempt to prevent this, we have explored the possibility of coating silicone catheters with a new anticoagulant, lepirudin, which, because it is a protein, has a natural tendency to adsorb to polymeric surfaces. We have shown that lepirudin adsorbed to silicone retains much of its anti-thrombin activity, and that the drug is only partially displaced by plasma proteins. Therefore, it may be effective in reducing the incidence of thrombosis associated with these catheters.

Z01 CL010337-01

Title The Effect of Thyroid Hormone on Parameters of Hypercoagulability

Dates from: 10/01/2002 to: 09/30/2003

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

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

Collaborator, NIH F. Pucino, PharmD (PHARM, CC)

Total Staff Years .2

Human Research Human subject research

Keywords thyroid hormone, hypercoagulability

Summary Because of an increased incidence of thromboembolic disease associated with thyroiditis, thyroid hormone has been suspected of impacting the normal thrombohemorrhagic balance. However, the effect of the hormone has never been studied in the absence of inflammatory thyroid disease.

We have isolated the effect of thyroid hormone by studying it in patients with a history of thyroid cancer who had had total thyroidectomies. Blood samples were obtained from these patients when they were taking suppressive doses of thyroxin, and were therefore chemically hyperthyroid, and again after they had discontinued their replacement in order to have a I131 thyroid scan and were chemically very hypothyroid. A series of analytes related to coagulability and fibrinolysis were measured.

We found that when the patients were hyperthyroid, they had elevated levels of prothrombin fragment F1+2, factor VIII, and plasminogen activator inhibitor-1, all of which suggest that thyroid hormone is prothrombotic.

Z01 CL010338-01

Title Pharmacokinetics of Pulse-Sprayed Tissue Plasminogen Activator

Dates from: 10/01/2002 to: 09/30/2003

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

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

Collaborator, NIH Richard Chang, MD (DDR, CC)

Total Staff Years .2

Human Research Human subject research

Keywords tissue plasminogen activator, thrombolysis, fibrinolysis

Summary For several years, we have been treating deep vein thrombosis with recombinant tissue plasminogen activator that is injected directly into the clots. We have now collected blood samples before and at several points following the treatment to determine how long the drug has a systemic effect. We found that it circulates in an active form for only 1 to 2 hours after injection and then becomes inhibited by a rise in plasminogen activator inhibitor-1.

Z01 CL010339-01

Title An Analysis of Laboratory Tests for Heparin-Induced Thrombocytopenia

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborators, Lab Paula K. Merryman (HEME, CC)
Khanh L. Nghiem (HEME, CC)

Collaborators, Extramural Vera Malkovska, MD (Washington Hospital Center)
Jose Mendoza, MD (Washington Hospital Center)

Total Staff Years .3

Human Research Human subject research

Keywords heparin, thrombocytopenia, serotin-release

Summary Heparin causes thrombocytopenia in 1 to 3 percent of patients and leads to thrombosis in about half of these. Several laboratory tests can diagnose this problem, but none of them is perfect and some are very labor-intensive. Recently, a new assay method has been published that offers the promise of high sensitivity and specificity and low labor. In order to collect a sufficient number of patient samples to study this new assay, we have collaborated with physicians at Washington Hospital Center, where heparin-induced thrombocytopenia (HIT) is much more common than here. Forty patients are being accrued with a spectrum of clinical probability of actually having HIT. Other patients are being accrued at the Clinical Center. Samples from these patients will be tested with the new laboratory test for HIT, as well as the time-honored but difficult assay that we currently consider the best. If the new test performs well, we will discontinue our older method.

Z01 CL010340-01

Title Shell Vial Assay for Culture of Vaccinia Virus

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Daniel Fedorko, PhD (DLM, CC)

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

Collaborator, Lab Jeanne S. Preuss (DLM, CC)

Collaborator, NIH Jeffrey Cohen, MD (MVS, NIAID)

Total Staff Years .15

Human Research Neither human cells nor tissues

Keywords vaccinia, culture

Summary Diagnosis of smallpox vaccination complications may be difficult in some patients because signs and symptoms may resemble other infectious (e.g., varicella-zoster virus, herpes simplex virus) or noninfectious causes (e.g., allergic dermatitis, drug rash illnesses). To support two Clinical Center protocols, we developed a rapid-shell vial culture assay for detection of vaccinia virus in patient specimens. The Wyeth strain of vaccinia virus grew equally well at 35°C for 24 and 48 hours in HeLa 229, MRC-5, A549, Mink Lung, HEp2, Vero, and RhMK cells, but grew very poorly in CHO cells. Centrifugation (3,500 x g for 15 minutes at 25°C) of the inoculum onto shell vial HeLa 229 cell monolayers increased the sensitivity of the assay by 100 fold compared with uncentrifuged vials. Sonication of the inoculum improved virus recovery compared with an unsonicated virus suspension. We were able to detect infectious virus in the culture medium of shell vials at 24 hours post-infection, and we detected 100-fold higher virus titer in the culture medium of shell vials at 48 hours post-infection. Fixation of cell monolayers before antibody staining did not inactivate the vaccinia virus. We conclude that the shell vial assay for culture of vaccinia virus with specimen sonication and inoculum centrifugation should be useful for rapid and sensitive diagnosis of complications due to smallpox vaccination. This work was presented at the 19th Annual Clinical Virology Symposium, Clearwater, Florida, April 27–30, 2003. A manuscript has been prepared and submitted for publication.

Z01 CL010341-01

Title Fluoroquinolone Resistance Among Isolates of *Streptococcus pyogenes*

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Daniel Fedorko, PhD (DLM, CC)

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

Collaborator, Lab Nancy A. Nelson (DLM, CC)

Collaborators, Extramural Steve Yan, PhD (FDA)
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Total Staff Years .06

Human Research Neither human cells nor tissues

Keywords *Streptococcus pyogenes*, group A streptococci, fluoroquinolones, resistance

Summary We previously described fluoroquinolone resistance as an isolate of *Streptococcus pyogenes* isolated in our laboratory from the blood of an NIH patient (Yan, S.S., M.L. Fox, S.M. Holland, F. Stock, V.J. Gill, and D.P. Fedorko. 2000). Resistance to multiple fluoroquinolone antibiotics in a clinical isolate of *Streptococcus pyogenes*: Identification of the gyrA and parC genes and specification of the point mutations responsible for the resistances. *(Antimicrob. Agents Chemother.* 44: 3196–3198.) In the current study, we will determine the frequency of fluoroquinolone resistance among *S. pyogenes* isolates from Chicago. We will sequence the gyrA and parC genes of these isolates to determine the point mutations responsible for the fluoroquinolone resistance. Isolates are screened for resistance using a panel of antibiotic disks (Kirby-Bauer method), and the resistance is confirmed with a minimum inhibitory concentration method (eTest). Among 93 isolates, we identified 10 with varying degrees of fluoroquinolone resistance. We are screening another 89 isolates. All resistant isolates will be sequenced using the primer sets described in our previous publication.

Z01 CL010342-01

Title Evaluation of Laboratory Diagnostic Methods

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Glen Hortin (DLM, CC)

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

Collaborators, Lab Gyorgy Csako, MD (DLM, CC)
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Alan T. Remaley, MD, PhD (DLM, CC)

Total Staff Years 1.2

Human Research Human cells or tissues

Keywords diabetes, kidney disease, heart disease, plasma proteins, mass spectrometry

Summary Goals of this project are to improve clinical laboratory methods for diagnosis of disease. Studies include analysis of clinical laboratory practices, analysis of the accuracy of laboratory tests, and development of new tests and testing technologies. The data from a national survey of laboratory practices for measurement of parathyroid hormone during surgery on parathyroid glands were evaluated and published. Data from a multicenter evaluation of the performance of glucose meters used by diabetics to measure their blood sugar levels were evaluated. This study, which provides an extensive evaluation of the consistency and accuracy of blood glucose meters, will be useful for establishing goals for their performance and improving their accuracy. Results are in press. A variety of new approaches for the diagnosis of kidney disease were reviewed in a published editorial and ongoing research is seeking to improve the measurement of creatinine and to improve the diagnosis of kidney disease by laboratory methods. Mass spectrometry methods are under evaluation as a new approach for analysis of blood and urine specimens. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is used to analyze complex patterns of proteins and peptides in biological fluids. Results of our studies suggest that this method will be useful for studying a number of the small protein components of high-density and low-density lipoproteins (HDL and LDL). These studies may provide new insights into the structure of HDL and LDL, which are commonly measured in the laboratory to assess risk for cardiovascular disease. These studies also may identify new technologies for routine diagnostic methods. Ongoing studies are examining the interactions of the amino acid homocysteine with plasma proteins in an extension of previously published studies that examined relationships between homocysteine, cysteine, and plasma proteins. Increased concentrations of homocysteine are related to heart disease and abnormal formation of blood clots, so there is a need for better understanding of interactions of this amino acid. Other studies have been directed at improving the measurement of the activity of proteases, which are involved in many important processes, such as blood coagulation. New reagents have been developed for measuring protease activity.

NURSING DEPARTMENT

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Z01 CL001131-04

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Margaret F. Bevans (NURS, CC)

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

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Collaborators, Extramural Nancy Kline Leidy, PhD (MEDTAP International)
Karen Soeken, PhD (University of Maryland)

Total Staff Years .08

Human Research Human subject research: Interviews

Keywords non-myeloablative bone marrow transplant, myeloablative bone marrow transplant, bone marrow transplant, quality of life, symptom distress

Summary Clinical research in blood stem cell and bone marrow transplantation documents improvements in disease-free intervals, disease-free survival, and the severity of treatment-related toxicities. However, it is important for patients and families to know the quality of life (QOL) they can expect following an allogeneic transplant. The purpose of this study is to describe the QOL experienced by patients undergoing a non-myeloablative allogeneic peripheral blood stem cell transplant and compare it with the QOL experienced by patients undergoing a myeloablative transplant. Patients must be over the age of 18 to enroll. Using touch screen computers, patients respond to questionnaires that measure QOL and symptom distress. 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 for currently enrolled subjects. The protocol is closed to new subjects.

Z01 CL001132-04

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

Dates from: 10/01/2002 to: 09/30/2003

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

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

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Collaborators, Extramural Nancy Kline Leidy, PhD (MEDTAP International)
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Total Staff Years 1.54

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 multidrug regimens known as highly active antiretroviral therapy (HAART), HIV infection now can 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 versus continuous HAART in the treatment of HIV disease. Adult HIV patients in the outpatient clinic completed questionnaires measuring QOL and symptom distress using touch screen computers seven times over an 88-week period. Data collection for this study is completed. Several analyses have been conducted and presented at two national meetings. A manuscript is under development.

Z01 CL001133-04

Title	Quality of Life in Melanoma Patients Receiving Vaccine or with Interleukin-2
Dates	from: 10/01/2002 to: 09/30/2003
Lead 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)
Collaborators, NIH	Douglas Schwartzentruber, MD (NCI) Steven Finkelstein, MD (NCI) Claudia Seip, RN (NCI)
Collaborators, Extramural	Nancy Kline Leidy, PhD (MEDTAP International) Karen Soeken, PhD (University of Maryland)
Total Staff Years	.9
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 except 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 on 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 three times: prior to, during, and post-therapy. Data will be analyzed using multivariate techniques. To date, 101 subjects have been accrued. Data collection and subject accrual continue.

Z01 CL001134-04

Title Quality of Life in Patients with Heart Disease and Left Ventricular Dysfunction

Dates from: 10/01/2002 to: 09/30/2003

Lead 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)

Collaborator, Extramural Nancy Kline Leidy, PhD (MEDTAP International)

Total Staff Years .16

Human Research Human subject research: Interviews

Keywords heart disease, left ventricular dysfunction, quality of life, symptom distress, angina

Summary Most 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 relationship between underlying cardiac condition, anginal symptoms, symptom distress, and health-related quality of life (HRQL) in patients with chronic ischemic heart disease and left ventricular dysfunction. The trend in HRQL across time versus treatment group (medical or surgical management) also was 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 parameters (viability). Questionnaires were administered three times over a 1-year period. Data collection is completed. Data analysis is ongoing.

Z01 CL001135-03

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

Dates from: 10/01/2002 to: 09/30/2003

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

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

Collaborator, NIH Lameh Fananapazir, MD, FRCP (CB, 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 life-saving 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 mailed questionnaire measuring HRQL, illness perceptions, and symptom distress. Structural equation modeling techniques will be used to analyze data. Data collection for this study continues.

Z01 CL001136-02

Title Pain and Palliative Care Evaluation Study

Dates from: 10/01/2002 to: 09/30/2003

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

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

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Donna Pereira, MA, RN (CC)
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Total Staff Years .1

Human Research Human subject research

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

Summary This is a randomized, repeated measure 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 participating in the National Cancer Institute's Surgery Branch protocols are asked to participate in a pain- and symptom-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. Forty-six patients have been enrolled in the study this year. Preoperatively, 23 patients were randomized into the control arm (standard care) of the study, while 23 patients were randomized to the treatment arm (pain and palliative care consult). Four patients from the control group were crossed over to the treatment group at the discretion of their attending physician. A total of 98 patients have been accrued to date. Data collection and subject accrual continue.

Z01 CL001137-02

Title Constipation in Pediatric Cancer Patients

Dates from: 10/01/2002 to: 09/30/2003

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

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

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Total Staff Years .3

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, but it has never been tested in the pediatric population. A pilot study using the CAS tool in children diagnosed with cancer is being conducted. Eleven participants have been accrued to date. Data collection and subject accrual continue.

PHARMACY DEPARTMENT

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1 Z01 CL005093-02

Title Influence of MDR-1 Genotype on Indinavir and Saquinavir Pharmacokinetics

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Scott R. Penzak, PharmD (CC)

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

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

Collaborators, NIH Gaetane Brunson, RN (NIAID)
Judith Falloon, MD (CMRS, NIAID)
Elizabeth Formentini (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 multidrug 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 a person's particular type of MDR1 gene (his or her genotype) influences the extent that P-gp limits the absorption of HIV protease inhibitors. The purpose of this study is to see how the MDR1 genes that each person got from his or her parents might affect how well he or she 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 (doses), and blood will be collected afterward to see whether MDR1 genotype influences the blood levels of these HIV medicines. Study subjects also will 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 Institutional Review Board for initial review on September 18, 2002. To date, 18 subjects have been enrolled in this study; the relationship between midazolam concentration and indinavir pharmacokinetics has been assessed in 11 of the subjects. CYP3A phenotype, determined using a single midazolam concentration, significantly correlated with indinavir oral clearance ($r = 0.7$; $p = .016$) and exposure ($r = 0.644$; $p = .033$) in these individuals. In addition, indinavir oral clearance was significantly higher in subjects with midazolam concentrations below, versus above, the median midazolam concentration (0.436 vs. 0.238 L/hr/kg; $p = .0019$). While these results are ancillary to the primary objective of this investigation, they are the first to show a significant relationship between CYP3A phenotype and protease inhibitor pharmacokinetics.

1 Z01 CL005095-02

Title Valganciclovir Pharmacokinetics

Dates from: 10/01/2002 to: 09/30/2003

Lead 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 is providing 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 after organ transplant that can result in significant illness or death. To date four male and two female kidney transplant recipients (three cadaveric and three living donor transplants) 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 900 mg orally per day. These two phases will be compared for equivalency of drug levels and exposure. The third phase consists of serial blood levels following 450 mg of oral valganciclovir daily. The fourth phase consists of serial blood samples after 1 g of oral ganciclovir every 8 hours. The third and fourth phases will be compared for equivalency of drug levels and exposure. At this time, all four phases are complete in three subjects, one subject completed three out of four phases, and another subject completed the first phase. Since the blood samples will be pooled and processed after seven patients have completed all four 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 also will be useful as a foundation to study the pharmacokinetics of valganciclovir in transplant patients with compromised renal function.

1 Z01 CL005096-01

Title Prednisolone Pharmacokinetics in the Presence and Absence of Ritonavir

Dates from: 09/01/2003 to: 09/30/2003

Lead Investigator Scott R. Penzak, PharmD (CC)

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

Collaborators, Lab Christine Y. Hon, PharmD (CC)
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Total Staff Years .2

Human Research Human subject research

Keywords prednisone, prednisolone, ritonavir, corticosteroid, protease inhibitor, osteonecrosis, HIV, AIDS, cytochrome P450, pharmacokinetics

Summary Corticosteroid administration has been associated with a variety of toxicities, including osteonecrosis and Cushing's syndrome, in patients with HIV infection. The prevalence of these toxicities has led to speculation that protease inhibitors impair the cytochrome P450 (CYP) 3A4-mediated metabolism of corticosteroids, leading to an increase in their systemic exposure and toxicity. Despite the hypothesized interaction between protease inhibitors and corticosteroids, drug interactions between agents from these pharmacologic classes have not been formally investigated. The purpose of this study is to determine the impact of the HIV protease inhibitor ritonavir on the pharmacokinetics of prednisolone after administration of oral prednisone to healthy volunteers. Ten study subjects will receive a single 20 mg dose of prednisone before ritonavir exposure, and again after 3 and 14 days of exposure at 200 mg twice daily. Blood and urine will be collected over a 24-hour period after prednisone administration to determine prednisolone pharmacokinetics and renal elimination. Blood also will be collected to determine multidrug resistance genotype, which may influence prednisolone disposition. To assess subject adherence with the study protocol, predose blood samples to determine ritonavir concentrations will be collected during the study; pill counts also will be conducted. Prednisolone and ritonavir concentrations will be determined by validated high-performance chromatographic methods. Noncompartmental methods will be used to characterize prednisolone pharmacokinetics before and after ritonavir. Pharmacokinetic parameters will be compared using analysis of variance with post-hoc testing. Geometric mean data with 90 percent confidence intervals will be reported. This study will characterize, for the first time, the influence of an HIV protease inhibitor (ritonavir) on the pharmacokinetics of a corticosteroid medication (prednisolone). This study was reviewed by the National Institute of Allergy and Infectious Diseases Institutional Review Board (IRB) in August 2003. IRB approval is expected in September 2003 and subject enrollment will likely begin in October 2003.

Rehabilitation Medicine Department

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Z01 CL060011-24

Title Diagnostic Capabilities of Ultrasound on the Oropharynx and Larynx

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Barbara C. Sonies, PhD (SLPS, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Collaborator, Lab Gloria Chi-Fishman, PhD (SLPS, CC)

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

Total Staff Years .5

Human Research Human subject research: interviews, minors

Keywords ultrasound imaging, swallowing, speech, viscosity, head neck tumors, three-dimensional imaging

Summary The purpose of this project is to evaluate and develop a variety of clinical applications for noninvasive ultrasound imaging to the diagnosis and treatment of impaired swallowing and speech and to evaluate the oropharyngeal structures (tongue, palate, floor muscles, hyoid, larynx, pharynx) in both normal and abnormal populations. 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 the National Institute on Deafness and Other Communication Disorders and the 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 normals revealed that there were significant effects of the thickness of the bolus on hyoid motion. Age and gender differences also were found during swallowing that suggest anatomical variations, sensory acuity, and muscle force changes occur with normal aging that can affect 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.

Z01 CL060017-13

Title	A Rigid Body Database on Human Movement
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Steven J. Stanhope, PhD (BS, CC)
Supervisor of Record	Lynn Naomi Gerber, MD (RMD, CC)
Collaborators, Lab	Susan Bender (BS, CC) Thomas M. Kepple (BS, CC) Kelly Nelson, MS BS, CC) Karen L. Siegel (BS, CC)
Total Staff Years	1.7
Human Research	Human subject research
Keywords	human movement analysis, biomechanics, gait analysis, induced acceleration analysis

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

Z01 CL060042-16

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Barbara C. Sonies, PhD (SLPS, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Collaborator, Lab Gloria Chi-Fishman, PhD (SLPS, CC)

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

Total Staff Years .4

Human Research Human subject research: interviews, minors

Keywords swallowing, neurological conditions, dysphagia, videofluorography, ultrasound imaging

Summary We are using both ultrasound and videofluorography to examine the effects of disease and status of oral motor function on the following conditions: stroke, postpolio syndrome, cystinosis, Sydenham's chorea, and Sjögren's disease. Patients who were seen at the NIH for baseline studies from 2 to 15 years after their initial examination are being followed to determine the course of change in these functions. Videofluorographic and ultrasound swallowing studies are administered along with complete oral sensory motor function examinations. Data on corticobasal degeneration and apraxia of swallowing have been analyzed. We completed a study to determine the kinematic strategies 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. These data support a theory of motor performance that suggests that the deglutitive motor system is more flexible than previously known. A subset of patients with cystinosis who were seen in the late 1980s and 1900s are being reevaluated for the effects of cystagon and kidney transplantation on swallowing and oral motor function. To date, 50 subjects have returned for the study. Initial inspection of the data from these subjects indicates that older subjects with cystinosis and those with multiple medical conditions, such as diabetes, hypothyroidism, and elevated blood pressure, appear to be at greater risk of oropharyngeal and esophageal dysphagia. New signs of swallowing difficulty also are appearing in postpolio patients. These continuing studies are pointing to a progression of swallowing difficulties that place patients with various neuromotor conditions at risk for aspiration.

Z01 CL060052-06

Title Rehabilitation Medicine Department Screening Protocol

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Lynn Naomi 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, or deglutition, or experience chronic pain. The goal is to help patients achieve their maximal level of function in order to optimally perform everyday activities. The RMD screening protocol gives staff an opportunity to pilot new tests, techniques, therapeutic modalities, technology, or equipment that have very low or no risk. Such media may be commonly used in rehabilitation practice but are being tested at NIH in a population that is different from the traditional. This protocol is designed for pilot work, and data generated from the pilot projects may be used, for example, to generate a protocol or to assure investigators of the ease/usefulness of the assessment, technology, or equipment. The RMD screening protocol was used to pilot the following projects: (1) comparing the relationship between the 9-minute Run Test performed on a 50 m indoor corridor to a 220 m indoor track in 7- to 10-year-old healthy children; (2) optimization of orthotic treatment; (3) pilot study to measure navicular drop in persons with rheumatoid arthritis: interrater and intrarater reliability; (4) pilot study to examine the feasibility of using an Accelerometer to measure activity levels in pediatric patients with Osteogenesis Imperfecta; and (5) ultrasound of subcutaneous tissue and quadriceps musculature in normals.

Z01 CL060054-03

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Barbara C. Sonies, PhD (RMD, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

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

Collaborator, Extramural Christian Macedonia, MD (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 respiratory system, in the fetus with ultrasound imaging. Pregnant women who receive care at the National Naval Medical Center are selected randomly 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, track amniotic fluid flow, and evaluate 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 followed carefully 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 (15 to 39 weeks gestational age) in women ages 19 to 42. Significant findings included the progression of suckling and other ingestive behaviors from simple to complex. Fetal swallowing occurred primarily in the presence of oral-facial stimulation. We are finding significant variations in the development and function of the ingestive system between age-matched controls and at-risk cases.

Z01 CL060055-03

Title Task-Induced Physiological and Biomechanical Changes of the *in Vivo* Human Tongue

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Gloria Chi-Fishman, PhD (RMD, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Collaborator, Lab Sungheon Kim, PhD (RMD, CC)

Collaborators, NIH Alan S. Barnett, PhD (CBDB, NIMH)
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Carlo Pierpaoli, MD, PhD (LIMB, NICHD)
Robert A. Wesley, PhD (OD, NCI)

Collaborators, Extramural Calvin Hisley, PhD (University of Maryland School of Medicine)
Lucinda A. Pfalzer, PhD (University of Michigan–Flint)

Total Staff Years .18

Human Research Human subject research

Keywords tongue, *in vivo*, human, volumetrics, hemodynamics, magnetic resonance imaging, ultrasound, diffusion tensor

Summary This protocol uses three-dimensional (3D) magnetic resonance imaging (MRI), Doppler ultrasonography, and other advanced MRI techniques such as diffusion tensor MRI and tagging MRI to address several important issues. These include (1) the compressibility of the human tongue and its common, yet untested, reference as a muscular hydrostat; (2) task-induced interactions between lingual musculature and vasculature and region-specific vascular demands; (3) changes in lingual fiber orientation, length, and strain distribution as a function of contraction tasks; and (4) effect of normal aging and disease processes on lingual myoarchitecture. By quantitatively addressing these issues, this protocol will contribute to a better understanding of the functional biomechanical as well as myoarchitectural intricacies of the *in vivo* human tongue. Our MRI data continue to show an average of about 8 percent (max = 12 percent) increase in tongue volume during maximal oropharyngeal voluntary isometric contraction. Our major accomplishment during 2003 was the validation of the MRI volume measurements. Validation studies were completed on three *ex vivo* models (two human and one calf). In these studies, measurements were compared between the volume measurements made from traced/rendered tongue MR images and the physical tongue volume across the factors of tracer (two trained biomedical engineering students), MR scanner (GE vs. Philips), scan type (current photon density protocol vs. higher resolution parameters), and imaging plane (sagittal,

axial, and coronal). The physical volumes were determined from the excised tongues (excised after MR scanning of the whole head) using the water-displacement method. Results showed no significant difference between the traced and the physical volumes as a function of scanner, scan type, or tracer. Tracer difference was significant for the coronal and axial imaging planes but not for the sagittal plane (which is the plane for our current *in vivo* studies). Tracer training and segmentation practice were found to be crucial. With training and practice, mean volume measurement error (i.e., difference from the physical mean) ranged from 0.22 percent (+/- 2.53) to 1.13 percent (+/- 2.93) for the sagittal plane. In diffusion tensor MRI, we have encountered susceptibility artifacts. To overcome this problem, we are in the process of developing a fast sensitivity encoding pulse sequence (known as SENSE) for in vivo imaging. Using tagging MRI, however, we have been able to collect *in vivo* 2D and 3D data on task-induced changes in tissue strain maps. These data will supplement our current *in vivo* fast spin echo proton density MRI studies with dynamic information on regional strain distribution in lingual tissue and are likely to provide biomechanical insights into the mechanisms for tongue volume changes. Evidence from our blood flow studies continues to reveal a linear relationship between changes in intramuscular pressures resulting from varying degrees of muscle contraction and patterns of hyperemia. Further, different reperfusion patterns in functional tongue segments suggest regional specificity in intramuscular vascular demands. This appears to correspond with recent histological confirmation of region-specific fiber types in the intrinsic tongue muscles. In sum, this protocol will continue to evaluate the muscular hydrostat concept, elucidate the biomechanical intricacies of the *in vivo* human tongue, and provide insights into the biomechanical elements and limitations of rehabilitative lingual exercises in patients with weakened or compromised lingual function, as well as preventive tongue strengthening exercises in adults undergoing normal aging.

Z01 CL060056-03

Title Effect of Task on Oral Pressure Dynamics During Swallowing

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Gloria Chi-Fishman, PhD (RMD, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Collaborators, Lab 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 .09

Human Research Human subject research

Keywords pressure, oral, tongue, swallowing, dysphagia, head-neck cancer, myositis, neurological disorders

Summary The overall objective of this protocol is to better understand normal and abnormal oral-pressure dynamics and suprahyoid-infrahyoid muscle group coordination as a function of swallowing task. The specific goals are (1) to determine if oral-pressure properties differ during discrete versus rapid sequential swallows in healthy adults of different ages; (2) to determine how oral maximum isometric pressure and task-specific swallowing pressure patterns differ between healthy adults and patients with reduced tongue strength and dysphagia; (3) to determine via surface electromyography (sEMG) if and how the swallowing task affects suprahyoid and infrahyoid muscle activity coordination; (4) to determine the relationship between oral-pressure patterns and videofluoroscopic signs of dysphagia in patients; and (5) to identify from the patients' oral-pressure profiles indices for ability versus inability to use rapid sequential swallowing as a compensatory strategy in dysphagia rehabilitation. To date, we have studied 30 healthy volunteers and 6 patients with neurologic impairments. In 2003, our analysis focused on normal suprahyoid and infrahyoid muscle activity coordination. Our measurements included max, mean, and ending amplitudes; start-to-max and max-to-end slopes; area under the curve; total sEMG activity duration; and suprahyoid-infrahyoid temporal differences in activity onset, offset, and time of max amplitude. Repeated measures analysis of variance were performed on the data of 107 rapid sequential and 106 discrete swallows with Bonferroni-Holm adjustment of alpha for the main effects and Tukey-Kramer adjustment of p for qualified post-hoc comparisons. Results showed no significant task-induced differences in suprahyoid-infrahyoid timing coordination measures. In addition, for all swallowing tasks, max amplitude was greater for suprahyoid than for

infrahyoid sEMG responses ($p < .0001$). Rapid sequential swallows had steeper suprahyoid start-to-max slope, steeper infrahyoid max-to-end slope, higher suprahyoid and infrahyoid ending amplitude, and shorter suprahyoid and infrahyoid total sEMG activity duration than any discrete tasks ($p < .0001$ in each case). Subjects differed significantly across all variables assessed, especially for the rapid sequential swallowing task in max amplitude and area under the curve. At least for the rapid sequential swallowing task, our two oldest subjects (59.5 and 60.8 years old) collectively differed considerably from the others in selected measures (e.g., lower max/mean amplitude and reduced slopes), suggesting a trend for age-related effects. While the muscles targeted by this study showed a clear pattern of coactivation regardless of task nature, hooked-wire EMG studies of hyolaryngeal depressors are needed to fully understand task-specific suprahyoid and infrahyoid neuromuscular coordination during swallowing. Our finding of greater suprahyoid than infrahyoid maximal amplitude suggests greater suprahyoid peak tension, which, in turn, suggests recruitment of a greater number of motor units and a greater amount of energy for the muscle complex that plays a leading role in swallow initiation. It remains unclear if our finding of partial suprahyoid and infrahyoid muscle relaxation during cyclical drinking reflects a passive by-product of shortened task execution time or an active motor control strategy. Only if the latter is proven true by future data can we then interpret such a finding within the context of motor economy and efficiency in the use of energy resources.

Z01 CL060057-02

Title Morbidity Following the Diagnosis and Treatment of Patients with Breast Cancer

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Charles L. Mcgarvey (PTS, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Collaborators, Extramural Lucinda A. Pfalzer, PhD (University of Michigan–Flint)
Peter Soballe, MD (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 breast cancer patients 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, 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 or functional limitations, or describing methods for their control with measures before medical intervention and long-term follow-up. The proposed outcome of this 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 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 ability to perform routine activities of daily living [ADLs], i.e., grooming, bathing, dressing, driving an automobile, and, in some cases, returning to regular work, recreational, and social activities). Researchers will also be able to determine the level of impairment at which these patients have lost independence in function and to identify those patients at higher risk for the loss of independence in function (e.g., ADLs). 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.

Z01 CL060058-02

Title Morbidity Following Breast Cancer Treatment: A Prospective Study

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Charles L. Mcgarvey (PT S, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Collaborators, Extramural Lucinda A. Pfalzer, PhD (University of Michigan–Flint)
Peter Soballe, MD (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 breast cancer patients before and after medical and surgical treatment, compared with a control group of 160 women who undergo an 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 years after medical treatment). The proposed-outcome study will include (1) a 2-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 survey/questionnaire that measures 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 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 medical treatment (loss of ability to perform routine activities of daily living [ADLs] i.e., grooming, bathing, dressing, driving an automobile, and, in some cases, returning to regular work, recreational, and social activities.) Researchers will also be able to determine the level of impairment at which these patients have lost independence in function, identify patients at higher risk for the loss of independence in function (e.g., ADLs), and determine risk factors for loss of function and disability. The study will identify factors associated with the above problems and try to determine their relationship to those factors.

Z01 CL060059-01

Title Investigations in Discourse Processing

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Carol M. Frattali (CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Total Staff Years .2

Human Research Human subject research

Keywords discourse, prefrontal cortex damage, text comprehension, lexical ambiguity, inferential ambiguity

Summary This protocol is designed to acquire an understanding of the underlying cognitive mechanisms involved in various aspects of discourse processing. Most studies have focused on understanding the role of the prefrontal cortex in discourse processes, and specifically on the role of the cognitive mechanism of suppression that allows selection of appropriate meanings when information is ambiguous during text comprehension. A range of experimental online tasks have been developed to investigate the processing of lexical ambiguities, inferential ambiguities, thematic aspects of stories, and relational language concepts presented either in pictorial (visual analogue) or textual representations. This fiscal year, we have studied about 30 subjects with prefrontal cortex damage either from penetrating head injury, stroke, tumor resection, or frontotemporal dementia. Accomplishments this year include publication of our findings in the *European Journal of Neurology*; recent submission of two other manuscripts to *Brain and Language*; and publication of two abstracts, two posters, and five scientific presentations. Our findings support the role of the prefrontal cortex in context-sensitive suppression function as necessary for text comprehension. In addition, lateralization of prefrontal cortex function has been found, with left lateralization dominance for processing lexical ambiguities and right lateralization dominance for processing inferential ambiguities.

1 Z01 CL060060-01

Title Microdialysis/Acupuncture Needle to Assess Local Tissue Milieu in Active and Latent Myofascial Trigger Points

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Jay Shah, MD (RMD, CC)

Supervisor of Record Lynn Naomi Gerber, MD (RMD, CC)

Collaborator, NIH Terry M. Phillips, PhD (DBEPS, OD)

Collaborator, Extramural Jerome Danoff, PhD (George Washington University Medical Center)

Total Staff Years .4

Human Research Human subject research: cells or tissues

Keywords rehabilitation, microdialysis, myofascial pain, myofascial trigger points

Summary The purpose of this study is to explore the use of a novel "microdialysis/acupuncture" needle, designed, fabricated, and tested by the investigators, to determine if this device and technique can be used to sample the chemical milieu of the soft tissue. Specifically, it will be used to measure and compare the quantity of small substances (electrolytes, muscle metabolites, inflammatory mediators, neurotransmitters, cytokines, and arachidonic acid derivatives) in three groups of subjects using a standardized anatomical location (trigger point 1) of the upper trapezius muscle, an area that is recognized as the most common location of myofascial trigger points (MTrPs) in that muscle. These substances are believed to play a critical role in the biochemistry and pathophysiology of soft tissue pain. Subjects of both sexes will be drawn from a range of ages. Trigger point 1 is selected specifically to standardize the location of a sampling point in three carefully selected groups: (1) healthy subjects without neck pain who have no MTrPs identified by palpation bilaterally in trigger point 1 (no pathological process, no symptoms), (2) healthy subjects without neck pain in whom latent MTrPs are identified by palpation in trigger point 1 in one of the upper trapezius muscles (pathological process, no symptoms), and (3) healthy subjects complaining of neck pain of less than 3 months' duration with active MTrPs identified by palpation in trigger point 1 in one of the upper trapezius muscles (pathological process with symptoms). This is not a treatment study. Rather, the primary goal of this study is to learn whether this device and technique can successfully sample the chemical milieu in healthy subjects who (1) have pain and those who don't, (2) have MTrPs and those who don't, and (3) have active MTrPs versus those who have latent MTrPs. We have recruited 18 subjects (8 women and 10 men), ranging in age from 23 to 66 years old. With our microanalytical sampling technique, we have collected samples at trigger point 1 in the upper trapezius muscle from each subject without any untoward events. We have measured 8 of the 21 analytes successfully in nine (with three in each group) subjects, demonstrating that this technique is able to recover extremely small quantities (<0.5 microliters) of very small substances (<100 kilodaltons molecular weight) directly from soft tissue.

Z01 CL060061-01

Title	Airway Fluid Dynamics in the Developing Fetal Respiratory System
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	Jeri L. Miller, PhD (RMD, CC)
Supervisor of Record	Lynn Naomi Gerber, MD (RMD, CC)
Collaborators, Lab	A. Sarcone, RA, RDMS (RMD, CC) Barbara C. Sonies, PhD (RMD, CC)
Collaborator, Extramural	Christian Macedonia, MD (National Naval Medical Center)
Total Staff Years	.3
Human Research	Human subject research
Keywords	respiration, amniotic fluid, Doppler sonography, fluid dynamics, polyhydramnios, oligohydramnios, prenatal development

Summary

The aerodigestive system is composed of a complex of integrated anatomic structures that support both ingestive and respiratory physiologies throughout the human lifespan. The developmental origins of this system—in both its form and function—begin *in utero*, where prenatal morphologies and associated processes form the foundations for extrauterine survival at birth. When this prenatal development is disrupted, alterations to normal physiologic functioning may serve as antecedents of later neonatal respiratory distress, dysphagia, or upper gastrointestinal dysfunction. Further, premature birth, before upper-airway processes are fully established, may exacerbate many of these conditions. In turn, this may prompt a cascade of respiratory-related dysfunctions throughout postnatal life. It is therefore important to understand how the respiratory system develops, how mechanisms regulate normal emerging physiologic processes, and the events that predicate fetal and neonatal compromise. To do this work in the living human fetus, we apply a novel noninvasive ultrasound imaging and analysis method to measure the role of altered amniotic fluid exchange in developing prenatal respiratory and ingestive mechanisms. The key components of this protocol follow. (1) Extend the use of prenatal ultrasound sonography to detect and measure the developing upper aerodigestive region. (2) Characterize differences in ingestive versus respiratory fluid flow-related dynamics prospectively across maturation in normal fetuses and those with conditions that may influence airway development. Using spectral Doppler-derived fluid flow analyses, we will map across the spectrum of gestation the fluid dynamics of upper airway development and the factors that influence functional integrity of upper-airway amniotic fluid exchange. (3) Identify how deviations in amniotic fluid regulation within the upper-aerodigestive

system may be associated with fetal and neonatal morbidity and mortality, and the predictive utility of these indices in conditions such as oligohydramnios or polyhydramnios. This study (approved by the NIH Institutional Review Board [IRB] on August 5, 2003; National Naval Medical Center [NNMC] IRB approval pending October 9, 2003 is a collaborative effort with the NNMC, Bethesda, Childrens and Womens Health. The project uses a novel standardized 4-axis sonographic examination to quantify growth and respiratory-related fluid flow mechanics in the upper airway of the living human fetus. The use of this noninvasive ultrasound technique as part of the clinical prenatal examination will not only discriminate function at four upper airway sites (perinasal, oral, pharyngeal, and tracheal), but will provide estimates of amniotic fluid flow volumes, inspiratory-expiratory fluid flow velocities and durations, and Doppler waveform patterns associated with fetal breathing and ingestive processes. This provides a method to explore how deviations in amniotic fluid regulation may be associated with morbidity and mortality, and the predictive utility of these indices in understanding conditions such as oligohydramnios or polyhydramnios. The germinal database will include healthy fetuses 16.0 to 39.6 weeks gestational age and test cases with polyhydramnios/oligohydramnios. By elucidating how developing structures integrate with emerging upper respiratory behaviors, this work will document the maturational events underlying normal function at birth that in turn may facilitate future clinical strategies for successful postnatal care.

Transfusion Medicine Department

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Z01 CL002040-19

Title Significance of Anti-HIV Antibody in Asymptomatic Donors

Dates from: 10/01/2002 to: 09/30/2003

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

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Total Staff Years .37

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 159 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 159 WB-positive donors, 46 (25 percent) are alive and in active follow-up; 72 (45 percent) are dead, and 41 (26 percent) are lost to follow-up (LTFU); 13 of the 73 LTFU were known to have AIDS at the time they left the study. Of the 46 in active follow-up, 25 (54 percent) have had an AIDS defining event. Of the 72 dead, 61 (85 percent) died from an AIDS-related illness, 92 percent of which occurred before HAART therapy became available. In contrast, only five of 59 (8 percent) who survived long enough to receive HAART succumbed to HIV infection. There is a significant difference between the mean CD4 count at entry for those alive (510 cells/ml) and those dead (391 cells/ml) and the entry HIV RNA level (3.17 log copies/ml and 3.77 log copies/ml, respectively). There are three treatment-naive long-term (> 10 years) non-progressors (LTNP), none of whom were homozygous for the CCR5-delta-32 mutation. No factors have been identified that distinguish the LTNP from the rest of the cohort. In contrast to published studies, no survival advantage of coexistent GBV-C/HGV infection has been observed in this cohort. No evidence of HIV infection evolved in the initial anti-HIV positive, but WB-indeterminate or WB-negative subjects. Treatment with HAART therapy is being conducted by personal physicians or through other NIH protocols. In summary, this cohort of HIV-infected blood donors died largely of AIDS before the advent of HAART. The impact of HAART on those who lived to receive therapy has been dramatic.

Z01 CL002062-13

Title Treatment of Familial Hypercholesterolemia by Dextran Sulfate Apheresis

Dates from: 10/01/2002 to: 09/30/2003

Principal Investigator Susan Leitman, MD (DTM, CC)

Supervisor of Record Harvey J. Alter, MD (DTM, CC)

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

Total Staff Years .1

Human Research Human subject research: tissues, cells

Keywords hypercholesterolemia, apheresis

Summary Patients with familial hypercholesterolemia (FH) type IIa are at high risk of premature coronary artery disease due to elevated low density lipoprotein (LDL) and Lp(a) cholesterol levels. Diet and drug therapy can reduce cholesterol concentrations in most patients with heterozygous FH, but a small proportion of heterozygotes and nearly all homozygotes do not respond to therapy. Selective removal of LDL by dextran sulfate affinity adsorption was evaluated in these patients in a collaborative multicenter U.S. study. The dextran sulfate apheresis system (Liposorber LA-15, Kaneka, Japan) removed LDL and Lp(a) without lowering HDL or albumin levels, thus avoiding the need for colloid replacement solutions. Six FH patients were enrolled at the Clinical Center; the total cohort enrolled nationwide included 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 FDA 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-years 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 these two subjects is likely to be the oldest living survivor in the world with this disorder, and he has undergone biweekly apheresis therapy at the NIH for the past 25 years.

Z01 CL002064-12

Title Characterization of Newly Identified Viral Genomes and Their Clinical Correlation

Dates from: 10/01/2002 to: 09/30/2003

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

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

Total Staff Years .1

Human Research Human cells or tissues

Keywords viral genomes

Summary There are two components in this project and both extend from our continuous commitment to the clinical investigation of viral hepatitis. One of these is an effort to respond to the increasing demand for a more precise measurement of relevant genomic information in any viral infection. The knowledge of the presence of specific viral gene will help in identifying the infectious agent. However, a more precise and quantitative analysis of the specific gene is required 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. This previous research-oriented question can now 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 (HBV), hepatitis C virus (HCV), and HIV infection. Whenever possible, we improved the basic PCR technique to become a semi-quantitative procedure. During the last 2 years, we applied the same principles of using PCR as the 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 nontransfused controls. It could be found in patients with chronic infection with mild or no observed liver function abnormality, but their 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 always tried to find other causative 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 CRADA. We divided responsibilities by focusing our efforts on 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.

Z01 CL002068-12

Title A Prospective Study of Anti-hepatitis C Virus Positive Blood Donors

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborators, Lab Cathy C. Conry-Cantilena, MD (IDS, CC)
Cathy Schechterly, BA (IDS, CC)
James Waikuo Shih, PhD (IDS, CC)

Collaborators, NIH Jay Hoofnagle, MD (DDN, NIDDK)

Collaborators, Extramural Joan Gibble, MD (American Red Cross, Chesapeake)
Paul Ness, MD (Johns Hopkins University)

Total Staff Years .65

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 (*N Engl J Med* 334:1691,1996), and the trends have remained the same over time. Unexpected findings were the high proportion (41 percent) of RIBA-positive donors who admitted to prior (remote) intravenous drug use and the strong independent association between cocaine snorting and HCV positivity. Shared paraphernalia for snorting accompanied by epistaxis may serve as a covert vehicle for parenteral viral transmission. Among anti-HCV-positive/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 of 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.

Z01 CL002076-08

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

Dates from: 10/01/2002 to: 09/30/2003

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, HCV

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 designing and constructing immunogenic plasmids. This includes 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 usage of adjuvants or other immune modifiers. The third phase is to evaluate the immune responses, and the final phase is to conduct a protectivity study. Great efforts have been devoted to determine the immune response, including assay developments. The protectivity study had to be conducted in an appropriate animal model. To determine the optimal challenge, inoculums for HCV vaccine remain to be determined. Each of these phases requires many different components and the development, implementation, evaluation, and improvement steps would over-lap. In FY 95 and FY 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 problems related

to the vaccine development for hepatitis C. One major concern is the genetic instability of the infectious agent. There are two hypervariable regions in the putative HCV envelope proteins. Immune escape mutants have been attributed to mutations in these regions. Experimentally infected chimpanzees and HCV-infected patients have been found to repeat bouts of infection with either homologous or new strains of HCV. This failure to develop protective immunity links to the high chronicity rate in HCV infection. Directly inducing strong cell-mediated immunity, especially protective cytotoxic T lymphocyte responses, may not only help in preventing initial HCV infection, but may also serve as a mechanism for immune modulation to overcome existing infection. Using the mouse model, we evaluated 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 past years, we tested the genetic sequences of many HCV-related immunogens to establish the best candidate DNA vaccine. We 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. During the last period, we conducted studies combining several immunogens to evaluate their interaction or interference. We also developed boost strategy. Currently, we are evaluating our prime-boost procedures and formulations. 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.

Z01 CL002078-08

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

Dates from: 10/01/2002 to: 09/30/2003

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)
Patrizia Farci, MD (LID, NIAID)
Robert Purcell, MD (LID, NIAID)

Total Staff Years .45

Human Research Human subject research: 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 appears 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, which reflects the extent of viral diversity (the degree of sequence divergence within the viral quasi-species). We have found that a mean Hamming distance of 12 to 16 weeks after the onset of acute infection predicts whether the patient will recover from HCV infection or develop persistent infection and chronic liver disease. Patients who recover have a declining Hamming distance as antibody to HCV develops, signifying immunologic containment and then clearance of the virus. In contrast, the majority of patients demonstrate an increased mean Hamming distance as antibody appears. This suggests that if the immune response is not sufficient to clear the virus, it paradoxically exerts immune pressure that results in mutations (escape variants) that lead to persistent infection. Interestingly, patients with fulminant hepatitis have a very low degree of viral diversity because they succumb

to the infection before the immune system can clear the virus or exert immune pressure. This study has been published (*Science* 288:339-344. 2000). In the next phase of this study, we will 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 antigens function compared to patients who recover from acute HCV infection.

Z01 CL002079-07

Title Hepatitis C Virus Infection in Infants and Children

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, Lab Cathy A. Schechterly (IDS, CC)

Collaborators, Extramural Camilla Baxter, BS (Children's National Medical Center)
Naomi Luban, MD (Children's National Medical Center)
Parvathi Mohan, MD (Children's National Medical Center)

Total Staff Years .15

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. During this interval, 5,546 children who met eligibility criteria were transfused at CNMC. The mean age at transfusion was 1 year (range, birth to 10.7 years). Thus far, 2,668 children (49 percent) have been recalled and provided consent/assent. The mean age at testing was 11 years (range 4 to 17 years). Of the 1,753 children fully tested for antibodies to HCV and hepatitis G virus (HGV), 36 (2.0 percent) are anti-HCV positive and 100 (5.7 percent) are HGV RNA positive. The HCV and HGV prevalence in age-matched 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 may serve to shift emphasis to pediatric populations where response rates may be higher and the long-term benefit greater.

Z01 CL002080-08

Title Natural History of Hepatitis C Virus Infection

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, Lab Cathy Schechterly, BA (IDS, CC)

Collaborator, NIH Leonard Seeff (DDP, NIDDK)

Total Staff Years .2

Human Research Human subject research

Keywords hepatitis C, natural history, transfusion-associated hepatitis

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 multicenter 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 (statistically insignificant). There was a slight increase in liver-related mortality in the hepatitis group (3.3 vs. 1.4 percent = .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.

Z01 CL002082-08

Title Studies of Viral Hepatitis and AIDS in the Chimpanzee Model

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, Lab June Germain (IDS, CC)

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

Collaborators, Extramural Michael Busch, MD, PhD (Blood Centers of the Pacific)
Krishna Murthy, DVM (Southwest Foundation for Biomedical Research)

Total Staff Years .08

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 undetectable levels of HCV RNA by the most sensitive nucleic acid testing assays currently available for blood screening.

2) Viral Inactivation: In collaboration with Cerus Corp., the chimp model was used to establish the efficacy of psoralen/UV-inactivated platelets. This is the first viral inactivation procedure that maintains the integrity of the cellular components of blood. Three chimpanzees have 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 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.

Z01 CL002085-08

Title Methods for Positive and Negative Selection of Hematopoietic Progenitor Cells

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Elizabeth J. Read, MD (CELL, CC)

Supervisor of Record Harvey J. Alter, MD (DTM, CC)

Collaborators, Lab Charles S. Carter (CELL, CC)
Hanh M. 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 .7

Human Research Human subject research: cells and tissue

Keywords hematopoietic progenitor cells

Summary Preclinical and clinical studies of automated closed systems for positive and negative selection of lymphohematopoietic cells have been conducted 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.: I*solex studies of the automated Isolex 300i for immunomagnetic selection of hematopoietic progenitor cells were completed. Over 100 selection procedures on version 2.0 (either positive only or combined positive/negative selection) have been completed, and over 100 selection procedures on version 2.5 were completed over the past 3 years. Studies of combined positive/negative selection aimed at 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 peripheral blood stem cells using this system. The results showed a mean CD34+ cell recovery of 55 percent and a mean CD3+ cell depletion of 5 logs. This system may be incorporated into future clinical trials.

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 will be continued into FY2004, and will serve as the foundation for clinical trials of *ex vivo* generated angioblasts for treatment of coronary artery and myocardial disease. In FY2003, additional AC133 selection studies were performed on the Miltenyi CliniMacs system and plans were made to expand these studies in the coming fiscal year to support cardiology cell therapy initiatives.

Z01 CL002089-07

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Susan Leitman, MD (DTM, CC)

Supervisor of Record Harvey J. Alter, MD (DTM, CC)

Collaborator, Extramural Jaime Oblitas (NIH, MT)

Total Staff Years .1

Human Research Human subject research: 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 -timulating 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. Sixty-five Clinical Center patients have received G-CSF-mobilized granulocytes. Forty-three were profoundly neutropenic, including 20 patients with severe aplastic anemia (SAA), 14 stem cell transplant recipients, eight patients with lymphoma/leukemia, and one with breast cancer. The remaining 22 patients had CGD. In the neutropenic patients, 26 had systemic filamentous fungal infections, 14 had bacterial infections, two had candidemia, and one had RSV infection. The mean increment in granulocyte count 1-hour post-transfusion was 2600/µL, and counts greater than 500/µL above baseline were sustained for 12 to 24 hours. Two of the 21 neutropenic, immunosuppressed patients who survived longer than 2 weeks after the initiation of granulocyte transfusions developed HLA allosensitization, as did 2 of the 15 CGD patients. In the absence of HLA allosensitization, granulocyte transfusions were associated with progressive hypoxia, pulmonary infiltrates, and an ARDS-like event in four of 20 SAA patients, versus 1 of 21 CGD patients. Of the neutropenic patients with tissue molds, 13 of 26 stabilized or improved during granulocyte transfusion therapy, but only eight of 26 survived hospitalization. In contrast, seven of 14 with bacterial processes were discharged from the hospital. Nineteen of 22 patients

with CGD had resolution of their fungal (ten of 13) 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.

Z01 CL002091-07

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Susan Leitman, MD (DTM, CC)

Supervisor of Record Harvey J. Alter, MD (DTM, CC)

Collaborators, Extramural Dennis Confer, MD (NMDP)
Megan Lubitz, RN (Marrow Donor Center)

Total Staff Years .1

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 May 31, 2003, 5.1 million donors were participating in the registry and more than 15,000 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 GVHD, and disease-free and overall survival. As of September 30, 2003, 84 NIH donors had undergone 103 apheresis procedures to collect PBSCs for unrelated NMDP recipients. There were 67 of 84 (80 percent) who required only a single apheresis procedure to collect an adequate cell dose for transplant, while 17 of 84 (20 percent) had a poor CD34 mobilization response to filgrastim and needed two consecutive apheresis procedures to collect an adequate cell dose. There were five females of the 84 donors who required a central line. All donors experienced 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 40,800/ µL, and postapheresis

thrombocytopenia (less than 100,000/ μL) occurred in 11 of 84 donors (13 percent), 9 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 because neither anesthesia nor hospitalization was needed; 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 one year is not different among 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).

Z01 CL002095-07

Title Structure and Function of Granulocyte Antigens

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record Harvey J. Alter, MD (IDS, 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 NB1 glycoprotein (gp), which is expressed on subpopulations of neutrophils and encoded by the CD177 gene. The gene encoding the NB1 gp was recently sequenced and called NB1. Another group described a gene that they called PRV-1 that is highly homologous to NB1. PRV-1 is overexpressed in neutrophils from patients with polycythemia rubra vera. The gene encoding PRV-1 differs from NB1 at four nucleotides. Previous work from our laboratory found that searches of human genome databases suggested that NB1 and PRV-1 were the same gene located on chromosome 19q13.2. In addition, database searches revealed that a pseudo gene homologous to exons 4 through 9 of PRV-1 was located on chromosome 19q13.2. The aim of these laboratory investigations was to determine if PRV-1 and NB1 are alleles of the same gene and if other genes homologous to PRV-1 are present in the human genome. The coding region of PRV-1 was amplified from human fetal liver total RNA by PCR, cloned into a TOPO plasmidand was used as a probe to screen a human bacterial artificial chromosome (BAC) library prepared with somatic DNA from a single individual (RPCI-11). Five BACs in the RPCI-11 library were reactive with the PRV-1 probes. The BACs identified in the library were analyzed by PCR and sequencing. Sequencing of the 5' and 3' termini of the BACs found that all 5 were located on chromosome 19, and 4 of the 5 had the same 5' and 3' termini sequences. PCR analysis of all 5 BACs rendered an amplicon when amplified with a primer pair encompassing PRV-1 exons 1 and 2 and a second primer pair encompassing exons 7 and 8. Sequencing revealed that both amplicons in all 5 BACs contained sequences homologous to PRV-1. In all 5 BACs the fragment containing exons 1 and 2 provided the same unique sequence that was identical to PRV-1. The fragment encompassing exons 7 and 8 proved to be two fragments, with similar but heterozygous sequences,

consistent with the presence of a pseudogene. One BAC containing PRV-1 was used in a fluorescence in situ hybridization (FISH) assay to localize genes homologous to PRV-1 within the human genome. FISH analysis showed that genes homologous to PRV-1 are located only on chromosome 19q13,2. These results show that PRV-1 and NB1 are alleles of the same polymorphic gene, CD177, which is located on chromosome 19q13.2. The coding region of CD177 from three healthy subjects was cloned and sequenced. Four of the six halpotypes were identical to PRV-1. The other two haplotypes each differed from PRV-1 at a single nucleotide. In conclusion, PRV-1 is the most common allele of CD177. The increased levels of neutrophil CD177 mRNA found in polycythemia vera are likely a marker of increased myelopoiesis in addition to a marker of myeloproliferation.

Z01 CL002097-05

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

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Elizabeth J. Read, MD (CELL, CC)

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Kenneth A. Hines, BS (CELL, CC)
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Collaborators, NIH Jay A. Berzofsky, MD, PhD (MIVRS, NCI)
Samir N. Khleif, MD (MB, NCI)
John Janik, MD (ID, NCI)
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John Charles Morris, MD (MB, NCI)

Total Staff Years .85

Human Research Human subject research: cells or tissues

Keywords dendritic cells, immunotherapy

Summary The goal of this project is to develop and evaluate methods for manufacturing dendritic cells (DCs) for clinical immunotherapy trials. In 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 FY2000, 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. In FY 2003, a pilot study evaluating the feasibility of DC cryopreservation was completed. Mature DCs generated by the method previously described were successfully cryopreserved in 5 percent DMSO and 6 percent Pentastarch, with 5 percent human serum albumin in Plasmalyte A. Higher protein concentrations did not significantly improve DC recovery after the freeze/thaw process. Additional cryopreservation studies are planned.

Z01 CL002099-05

Title The Use of Granulocyte Colony-Stimulating Factor in Healthy Donors

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record Harvey J. Alter, MD (IDS, CC)

Collaborator, NIH 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 Granulocyte colony-stimulating factor (G-CSF) is the standard growth factor for mobilizing hematopoietic progenitor cells in healthy peripheral blood progenitor cell (PBPC) donors. G-CSF is given daily for 5 days to PBPC donors prior to collect (PBPC) concentrates by apheresis. PBPC-concentrate donors given G-CSF experience splenic enlargement and, rarely, spontaneous rupture of the spleen. We evaluated the incidence and time course of splenic enlargement in PBPC concentrate donors and accessed factors effecting size changes. Healthy adults were given G-CSF (10 micrograms/kg/day) for 5 days and a PBPC concentrate was collected by apheresis. The day of apheresis and 4 days after apheresis, ultrasound was used to assess craniocaudal spleen length prior to giving G-CSF. Spleen length increased in 19 of 20 donors. On the day of apheresis, the average length was 14 percent greater than before G-CSF; 4 days after PBPCs were collected, spleen length was reduced in length, but remained greater than baseline levels. A second study was initiated to determine if spleen length returns to baseline levels 10 days after apheresis. Healthy adults were again given G-CSF (10 microgramsg/kg/day) for 5 days and a PBPC concentrate was collected by apheresis. Ultrasound was used to assess craniocaudal spleen length prior to giving G-CSF, the day of apheresis, and 10 days after apheresis. Spleen length increased in nine of ten donors. Mean spleen length changed from 10.7 cm pre-G-CSF to 12.0 cm on the apheresis day. The mean increase in length was 1.4 cm or 13.6 percent. Ten days after apheresis the spleen length fell to 10.7 cm and did not differ from baseline levels. For 8 of the 9 donors whose spleen length increased, the length was measured 10 days after apheresis and was within 0.5 cm of baseline length. In one donor, spleen length remained 0.7 cm or 7 percent longer than baseline length 10 days after apheresis. This donor was a 23-year old Caucasian female whose spleen length on the apheresis day was 1.9 cm or 18 percent longer than baseline length. We found that spleen length increases in PBPC donors are transient and reversible. In most donors the spleen returned to normal size within 10 days. Future studies will focus on the use of a new long-acting form of G-CSF, polyethylene glycolated-G-CSF (PEG-G-CSF), to mobilize PBPCs in healthy donors. The optimum dose and safety of PEG-G-CSF will be studied.

Z01 CL002103-04

Title Immune Therapy for Cytomegalovirus Infection

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator David Frank Stroncek, MD (LAB SS, CC)

Supervisor of Record Harvey J. Alter, MD (IDS, CC)

Collaborators, Lab Maria P. Bettinotti (LAB SS, CC)
Maurizio Provenzano, MD (LAB SS, CC)

Collaborators, NIH A. John Barrett, MD (NHLBI)
Scott Solomon, MD (HB, NHLBI)

Total Staff Years .5

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*2402 people, the immune-dominant peptide is pp65 328-337. We have found that peptide pp65 341-350 is immune-dominant for HLA-A*0101 and HLA-A*2402, and pp65 91-100 is immune-dominant for the antigen HLA-A*3301 and is an important Asian allele. In contrast, among individuals expressing HLA-A*03 there was no single immune-dominant peptide. Instead, three different pp65 peptides appear to be immune-dominant in different subsets of HLA-A*03 individuals. We have also begun collaborative studies with Drs. John Barrett and Scott Solomon of the National Heart, Lung, and Blood Institute. They are 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 donor's immune response to the vaccination. In a pilot study we found that it is possible to monitor the immune response to CMV by stimulating lymphocytes with pools of peptides 15 amino acids in length that overlap at 4 amino acids and measuring intracellular production of interferon-gamma.

Z01 CL002104-04

Title Molecular Testing Standards and New Amplification Approaches

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, NIH Richard Y. Wang (DTM, CC)

Total Staff Years .1

Human Research Neither human cells nor tissues: cells or tissues

Keywords molecular testing standards

Summary There are two components of 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 CRADA. The end point for this collaboration is to develop a prototype test and to demonstrate its utility with clinical specimens. We have constructed a particulate HCV internal standard (IS) based on murine amphotropic retrovirus. To achieve this, we went through a stepwise process including mutating HCV genome by inserting a 36 nucleotide base at nt 272 position in the 5'-UTR and then creating a retroviral vector clone pXT-HCV-NCC-D8 containing 948 bases of the HCV sequence. This vector was used to transfect a retrovirus packaging cell line, PA317. From the transfected cells, G418-resistant recombinant retrovirus producer clones were established and further characterized. Using sequence specific primers, we were able to show that HCV sequence-containing particles were produced. Both wild-type clones with HCV sequence and mutant clones with additional inserts were prepared and isolated. We were able to determine the insertion sequence length as expected by gene analyzer. One preparation of virus supernatant from a high virus producer, D8-54, was evaluated extensively. The relative copy number per milliliter was determined by different methods, including RT/PCR titer, electron microscope particle counting, infectious colony-forming counts, and end-point infectious titer. We found consistent results with different methods of determination, demonstrating that the approach could provide ideal particulate IS for HCV. We applied this IS to a small-scale study with clinical specimens and also developed 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—finding new amplification approaches—n a 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 thousand-fold amplification was demonstrated by estimating the probe products. Specificity was shown by a narrow range of strain-specific recognition. A U.S. patent was filed jointly by the University of Maryland and NIH based on these observations and is currently pending. To commercialize this patent, an industry partner capable of developing this technology was sought. Medical Analysis System of Camerillo, CA, 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 in our effort to find a manufacture for licensing purpose.

Z01 CL002105-04

Title Characterization of Human Pathogenic Mycoplasma from HIV-Infected Patients

Dates from: 10/01/2002 to: 09/30/2003

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

Collaborator, NIH Richard Y. Wang (DTM, CC)

Collaborator, Extramural Shyh-Ching Lo, MD, PhD (AFIP)

Total Staff Years 0

Human Research Human cells or 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 patients with AIDS or sexually transmitted diseases (STDs), 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 could not 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 4.8 percent of the cases

and 5.2 percent of the controls tested positive for *M. fermentans*-specific antibodies before 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, there is no serological evidence that suggests infection by *M. fermentans* is associated with development of GWI. We also studied blood, urine, oral swabs, and rectal swabs for evidence of mycoplasmal infection by culture from a group of 149 Gulf War veterans who complained of various illnesses and were enrolled in the second phase of the health evaluation by the Army Comprehensive Clinical Examination Program (CCEP). None of the urine samples, oral swabs, or rectal swabs grew *M. fermentans*. No mycoplasma organism was isolated from any of the 149 blood samples. PCR study was conducted using an 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 have limited our effort to the advising and technical support level.

Z01 CL002107-04

Title Prospective Studies of Phlebotomy Therapy in Hereditary Hemochromatosis

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Susan Leitman, MD (DTM, CC)

Supervisor of Record Harvey J. Alter, MD (DTM, CC)

Collaborator, Lab Yu Ying C. Yau, RN (DTM, CC)

Collaborators, NIH Charles Bolan, MD (DTM, CC)
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Total Staff Years .2

Human Research Human subject research: cells or tissues

Keywords phlebotomy therapy, hereditary hemochromatosis

Summary Persons with hemochromatosis constitute a plentiful and willing source of blood for transfusion. Recent changes in federal regulations have eased restrictions on the use of blood from hemochromatosis subjects for transfusion; however, no data exist to guide the performance of phlebotomy therapy in a blood donor center setting. We established and evaluated a program for treating persons with hemochromatosis in a donor center, and making their blood available for transfusion. Phlebotomy therapy was performed free of charge regardless of whether subjects met criteria for allogeneic donation. Hemoglobin of 12.5 g/dL was used as the threshold for performing phlebotomy and decreases in the MCV were used to guide the endpoints of therapy. One hundred thirty subjects were consecutively enrolled: 74 percent were homozygous for the C282Y mutation in the HFE gene, 76 percent met eligibility criteria for allogeneic donation, and 55 percent were previous blood donors. A median of 20 weekly or biweekly phlebotomies (range 7-99) were performed before the MCV reached the targeted endpoint of 3 percent below baseline, at which time the ferritin was less than 30 mcg/L and the transferrin saturation less than 30 percent. The median phlebotomy interval necessary to keep the MCV at this level during maintenance therapy was 10 weeks. No incident seroconversions for agents of transfusion-transmissible disease occurred during 1,402 donations. All subjects testing positive for viral agents gave a prior history of deferrable risk. As of September 2003, hemochromatosis donors were contributing 15 percent of the red cell units collected for allogeneic use in the NIH Clinical Center. Due to the steady contributions of NIH hemochromatosis donors, it was not necessary to postpone surgery or acquire blood from outside suppliers during periods of low inventory, at a time when regional blood supplies were at critically low levels in other hospitals. Our data demonstrate that hemochromatosis subjects can safely and significantly augment the allogeneic blood supply. Provision of phlebotomy therapy unrestricted by considerations of cost or suitability for

donation can improve access to care and remove incentives for incomplete risk disclosure. Current efforts are focused on critical examination of the role of double red cell donation by apheresis in the management of HH subjects, on optimizing the use of changes in red cell indices as a guide to the endpoints of phlebotomy therapy, on delineating the effect of phlebotomy therapy on arthritic symptoms, and on assessing changes in serum non-transferrin bound iron levels during therapy.

Z01 CL002108-04

Title Studies of ABO Incompatibility in Hematopoietic Stem Cell Transplantation

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Susan Leitman, MD (DTM, CC)

Supervisor of Record Harvey J. Alter, MD (DTM, CC)

Collaborators, NIH Charles Bolan, MD (DTM, CC)
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Total Staff Years .1

Human Research Human subject research: 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. The impact of ABO incompatibility on the outcome of hematopoietic transplantation is less well appreciated. In particular, nonmyeloablative conditioning regimens and peripheral blood stem cell grafts, which are the subject of intense investigation in several NIH protocols, may increase the risk of severe hemolytic complications due to ABO incompatibility between donor and host. Massive immune hemolysis caused by “passenger lymphocytes” in the stem cell graft, and pure red cell aplasia (PRCA) due to recipient anti-donor red cell isohemagglutinins, have occurred both more frequently and with greater clinical severity than that seen with myeloablative conditioning regimens and marrow-derived grafts. As a result of these events, the Department of Transfusion Medicine established procedures to monitor, evaluate, and implement management strategies for care of patients receiving ABO mismatched hematopoietic stem cell transplants at NIH. Serial daily laboratory testing, including complete blood counts, chemistries, direct antibody tests (DAT), and other red cell serologic assays were obtained in all lymphocyte-replete minor-ABO incompatible peripheral blood stem cell (PBSC) transplants. Significant hemolysis was observed in three of the first ten patients monitored in this fashion. Since inclusion of an antiproliferative agent in the GVHD prophylaxis regimen may affect the occurrence of hemolysis in this setting, transplant protocols were modified to include an antiproliferative agent, mycophenolate mofetil (MMF), as well as a calcineurin inhibitor (cyclosporine [CsA]) to prevent acute GVHD. Transplant outcomes in patients receiving CsA alone versus CsA plus MMF were followed prospectively. Thirteen patients received CsA alone, and 18 received CsA plus MMF. A modest effect on post-transplant serologic events was seen in the

CsA plus MMF group versus the CsA alone group, with weaker reactions involving donor ABO isohemagglutinins against recipient red cells and delayed disappearance of recipient-type red cells when MMF was used. These data were consistent with inhibition of isohemagglutinin production by MMF. However, there was no significant difference in the incidence of significant hemolysis between the two groups. Serologic detection of a positive DAT was poorly predictive of hemolysis, and most patients who manifested a positive DAT did not have hemolysis. To evaluate the effect of major ABO incompatibility on donor red cell engraftment following nonmyeloablative stem cell transplants (SCT), we compared transplant outcomes in patients receiving major ABO incompatible nonmyeloablative SCT (fludarabine/cyclophosphamide conditioning) with subjects receiving myeloablative SCT (cyclophosphamide/high-dose TBI). Donor red cell chimerism (detection of donor red cells in the recipient's blood) was markedly delayed following nonmyeloablative versus myeloablative SCT, median 114 versus 40 days, and correlated strongly with decreasing host anti-donor isohemagglutinin levels. Anti-donor isohemagglutinins declined to clinically insignificant levels more slowly following nonmyeloablative than myeloablative SCT (median 83 versus 44 days). Donor RBC chimerism was delayed more than 100 days in 9 of 14 (64 percent) and PRCA occurred in 4 of 14 (29 percent) patients following nonmyeloablative SCT, 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 nonmyeloablative SCT occurred significantly sooner in cases with, compared to those without, PRCA (30 versus 98 days). Patients with 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 red cell aplasia 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 nonmyeloablative SCT. Studies currently in progress are directed at determining whether clinical and serologic events following major ABO mismatched PBSC transplants are due to differences in the timing of plasma cell, B lymphocyte, and myeloid chimerism. Based on these studies, the DTM continues to evaluate optimal post-transplant monitoring protocols for management of minor and major ABO incompatibility between donor and recipient. Due to the poor predictive value of serial DAT testing, a modified approach will be implemented in upcoming and existing protocols using daily CBC and chemistry results with red cell serologic testing every 4 days.

Z01 CL002110-03

Title Transfusion-Related Infections Prospectively Studied (TRIPS)

Dates from: 10/01/2002 to: 09/30/2003

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Pamela L. Hernandez (IDS, CC)
Harvey Klein, MD (IDS, CC)
Cathy Schechterly, BA (IDS, CC)
James Waikuo Shih, PhD (IDS, CC)
Joni L. Trenbeath (IDS, CC)
Bernice L. Williams (IDS, CC)

Collaborator, NIH T. Cain, BA (CC)

Collaborator, Extramural Michael Busch, MD, PhD (Blood Centers of the Pacific)

Total Staff Years 4.17

Human Research Human subject research: cells or tissues

Keywords hepatitis, blood transfusion, adverse events, microchimerism, viruses

Summary Improved viral screening assays and more intensive questioning of donors for high-risk behaviors have resulted in dramatic declines in the rates of transfusion-transmitted hepatitis and AIDS. Nonetheless, there is a need for continued vigilance 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; (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 infectious 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 availability of

the repository will allow for the assessment of transfusion risk for newly emerging pathogens and also for known agents for which there is no practical assay currently available. For example, this would allow future testing for prions in new variant Creutzfeldt-Jakob disease (human variant of mad cow disease) or testing for the trypanosome that causes Chagas disease. Informed consent will be obtained to store and later test samples in the repository. Testing will be limited to infectious agents that potentially threaten the blood supply. No genetic testing will be performed.

Z01 CL002111-03

Title	RBC Leukocyte Reduction Filter Failures in Blood Donors with Sickle Trait
Dates	from: 10/01/2002 to: 09/30/2003
Lead Investigator	David Frank Stroncek, MD (LAB SS, CC)
Supervisor of Record	Harvey J. Alter, MD (IDS, CC)
Collaborators, NIH	Susan Leitman, MD (DTM, CC) Alan N. Schechter, MD (LCB, NIDDK)
Total Staff Years	.1
Human Research	Human subject research
Keywords	sickle cell trait, RBCs, leukocyte reduction filters

Summary

Fifty to seven-five percent of 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 is 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 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. We have shown that increasing the oxygen tension in sickle cell trait RBC components allows effective leukocyte reduction with a filter designed to remove leukocytes from RBCs. However, standard filters must be used with RBC components that are less than 24 hours old. To increase oxygen levels within 24 hours to levels sufficient to allow effective filtration, blood must be stored in gas permeable bags and agitated. Since it is likely that blood collection centers would have to document that rocking does not damage the RBCs, agitation is not practical for transfused blood. Alternatively, air can be added to blood storage bags to increase oxygen levels and allow effective filtration; however, it is not practical for blood collection centers to add air sterilely to blood components. We hypothesized that it would be possible to increase oxygen levels in sickle cell trait donor blood to sufficient levels to permit successful filtration without agitation by storage in gas permeable bags with a larger than normal surface-to-volume ratio and for a longer storage duration prior to filtration. At least one leukocyte reduction filter is available that can be used to remove leukocytes from blood stored up to 72 hours. When blood was filtered fresh it occluded these filters, but when it was stored 3 days in large capacity bags made from oxygen permeable material suitable for whole blood storage, CLX, oxygen tensions increased to levels high enough to allow effective leukocyte reduction by filtration.

Z01 CL002112-02

Title Citrate Effects and Magnesium Replacement During Large Volume Leukapheresis

Dates from: 10/01/2002 to: 09/30/2003

Lead Investigator Susan Leitman, MD (DTM, CC)

Supervisor of Record Harvey J. Alter, MD (DTM, CC)

Collaborators, Lab Charles Bolan, MD (DTM, CC)
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Collaborator, NIH Robert A. Wesley, PhD (OD, NCI)

Total Staff Years 1.1

Human Research Human subject research: cells or tissues

Keywords leukapheresis, apheresis, magnesium, hypomagnesemia, calcium, hypocalcemia, citrate

Summary Marked decreases in ionized magnesium levels occur during large volume leukapheresis; however, the role of magnesium supplementation has not been carefully studied in this setting. We performed a randomized, double-blind trial of intravenous magnesium sulfate administration in healthy allogeneic subjects undergoing peripheral blood progenitor cell (PBPC) donation. Thirty donors undergoing PBPC collection using standard citrate anticoagulant (ACD-A) and intravenous calcium prophylaxis were random-ized to receive either intravenous magnesium (0.2 mg magnesium per mL ACD-A) or placebo during the apheresis procedure. Seventy-five PBPC procedures were evaluated in these 30 subjects, 38 using placebo and 37 using magnesium sulfate. Group characteristics were the same for gender (eight men and seven women per group), weight (78 vs 81 kg), citrate infusion rate (1.36 vs 1.37 mg/kg/min), and volume processed (16 vs 17 liters). Serum ionized magnesium levels remained within the normal range with use of intravenous magnesium supplementation, but decreased to 39 percent below baseline with use of the placebo solution ($p < 0.0001$). Serum magnesium levels also decreased significantly following consecutive leukapheresis procedures in the placebo-, but not the magnesium-treated donors. Subjects receiving magnesium showed significantly more vigorous parathyroid hormone responses and higher glucose levels, and also tended to have higher serum potassium and ionized calcium levels than subjects receiving placebo. Mild paresthesias, coldness, and nausea occurred in 28, 20, and 7 percent of donors, respectively, with no significant differences between groups. During the study, severe citrate-related symptoms (chest tightness) occurred in only one subject (a donor receiving placebo infusions). We concluded that intravenous magnesium supplementation has a significant impact on serum magnesium levels and other metabolic parameters during large volume leukapheresis. Adverse clinical effects were rare in the presence of calcium prophylaxis. A much larger study would be required to detect small differences attributable to magnesium infusions. Based on this study, the DTM is re-evaluating guidelines for the use of magnesium supplementation during PBPC donation.

Z01 CL002113-02

Title Plasmapheresis of Anthrax Vaccinees for Production of Anthrax Immune Globulin

Dates from: 10/01/2002 to: 09/30/2003

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Human Research Human subject research: 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 have been previously available. This protocol provides 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 six months of the last dose if seven or more AVA inoculations were given. All volunteers were vaccinated as a requirement of their tour of duty and otherwise meet all blood donor eligibility criteria, in accordance with Food and Drug Administration (FDA) requirements and American Association of Blood Banks (AABB) standards. Plasmapheresis was accomplished using licensed apheresis devices and standard collection techniques, and products met all blood safety testing requirements mandated by the FDA. Plasma components collected under this protocol were stored in the frozen state as fresh frozen plasma (FFP) for possible administration to human patients critically ill with inhalational anthrax infection. Following collection of single-donor FFP units, subsequent plasmapheresis components were stored frozen in liter-bottles for Cohn-Oncley fractionation, by a commercial fractionator, into an immunoglobulin preparation suitable for intravenous use, 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 1/2 trial involving an Investigational New Drug (IND) exemption, with the IND held by the Centers for Disease Control

and Prevention (CDC). The plasma products collected under this protocol may also be used in pharmacokinetic, dose finding, and efficacy studies in animals, and to establish a repository of reference serum standards at the CDC. During the period from 4/30/2002 to 9/30/2003, 38 recently AVA-vaccinated DoD employees underwent plasmapheresis at NIH to donate hyperimmune anti-anthrax plasma. Subjects underwent an average of 5.2 apheresis donations each, performed at weekly to biweekly intervals, yielding a total of 200 plasmapheresis components. During each session, 700–900 mL of plasma were collected, based on the individual's weight and approved FDA Guidelines for plasma collection. Fifty-four 250–300 mL bags of Anthrax Immune Plasma (AIP) were shipped to the CDC National Pharmaceutical Stockpile. A total of 140 liters of hyperimmune plasma, stored frozen in liter bottles, were provided to a contracted commercial manufacturer for further preparation into AIGIV.

Z01 CL002115-01

Title Immunogenetics in Vaccine Therapy

Dates from: 10/01/2002 to: 09/30/2003

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Human Research Human subject research: cells or tissues

Keywords immunogenetics, polymorphism, vaccination, T cells, adaptive immunity, innate immunity, functional genomics, SNP detection, nasopharyngeal cancer

Summary The Immunogenetics Program of the Department of Transfusion Medicine is focused on the identification of genetic variables that may influence the outcome of immunization protocols against cancer and in general against diseases that are predominantly controlled by cellular immune responses. The Program includes two components: the Human Leukocyte Antigen (HLA) typing laboratory, which is mainly covering the routing identification of known genetic variance among different populations through HLA molecular testing. In addition the HLA laboratory will more broadly apply immunogenetic profiling that includes typing of other immune relevant genes such as Killer Cell Immunoglobulin Like Receptors (KIR), Cytokines, and FC-Receptors. The second component is the Immunogenetics Research Laboratory, which includes a Functional Genomics Unit, a Proteomics Unit, a single nucleotide polymorphism (SNP) detection unit, a pathogen chip detection unit, and a T cell physiology unit. All of these units and the HLA laboratory interact to develop an integrated approach to the study of individual responses to immunologic therapy aimed at the enhancement of adaptive or innate immune responses with special emphasis on cellular immune responses. Clinically, the Immunogenetics Program has developed two protocols in the last year. The first one is aimed at the recruitment of 30 Caucasian and 30 Chinese donors who will be tested for their immune responses to antigen (i.e., latent membrane protein-2 of the Epstein-Barr virus; LMP-2), or general pathogenic, or therapeutic stimulation with lipopolysaccharide (LPS) or interleukin-2 (IL-2). These responses will be compared to the genetic backgrounds of the two populations by comparing changes in global transcript and protein expression with different genetic

profiles that could be identified using a newly developed high-throughput oligonucleotide SNP detection chip. The hypothesis is that different ethnic backgrounds may modulate the response to general immune stimulators or antigen-specific responses in relation to differences in the ability of various genes to respond to stimulation. In addition, LMP-2 epitope mapping will be performed to see whether the notoriously different HLA phenotypes carried by the two populations lead to differences in T cell responses to one of the immunogenetic EBV proteins. This work will yield a global view of the possible influence that genetic background may have on disease predisposition, outcome, and response of therapy in the context of immune responses. In particular, it may explain differences in individual responses to T cell-aimed immunizations. The second clinical protocol developed during the last year is aimed at the immunization of patients with nasopharyngeal cancer (NPC) at high risk of recurrence after local control of the primary tumor. NPC is caused by EBV virus and is highly dependent on the expression of LMP-2. Our previous research in the context of metastatic melanoma has convincingly shown that immunization of patients with cancer with protein products derived from proteins expressed by cancer cells can reproducibly induce strong T-cell responses. These responses include the induction of large numbers of circulating CD8+ T cells capable of recognizing cancer cells in in vitro assays. However, the effectiveness of these cells in eradicating cancer in vivo remains disappointing. Improvements in clinical outcomes can be observed when immune stimulatory agents such as IL-2 are combined with the treatment. Therefore, this protocol will test whether: (1) immune responses can be elicited in patients with NPC using the LMP-2 protein from EBV that supposedly plays an oncogenic effect on the disease; and (2) these responses impact recurrence rates; (3) it will also estimate whether further studies are warranted to test whether the combination of immunostimulatory agents enhance the frequency of clinical responses in patients with advanced NPC. In conclusion, the Immunogenetics Program of the DTM, CC, is devoted to the development and implementation of translational medicine efforts in studying the immune responses in individuals of different ethnic background to active-specific immunization against cancer and other chronic conditions predominantly controlled by cellular immune responses.

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