Opportunities

Research and Training Programs 2002-2003
NIAID Division of Intramural Research

Opportunities

in the NIAID

Division of Intramural Research

National Institute of Allergy and Infectious Diseases
NATIONAL INSTITUTES OF HEALTH

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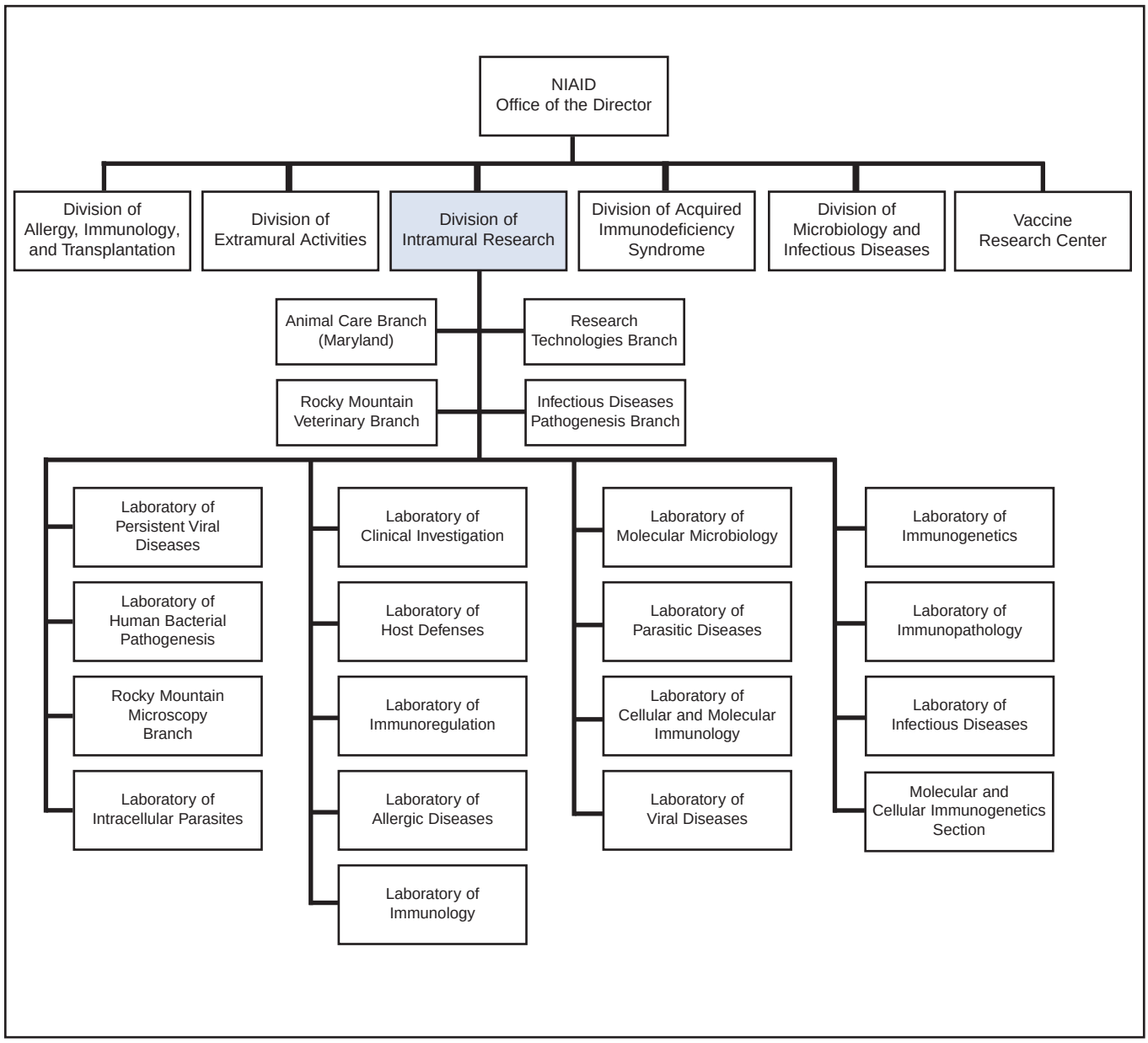
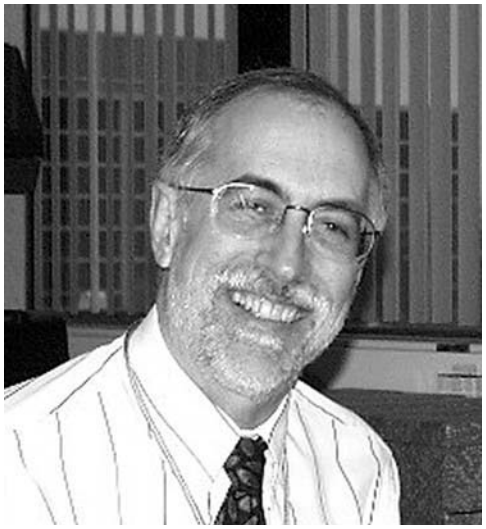
National Institute of Allergy and Infectious Diseases



Welcome to the DIR



Thomas J. Kindt, Ph.D.
Director
Division of Intramural Research, NIAID

Karyl S. Barron, M.D.
Deputy Director
Division of Intramural Research, NIAID

Welcome to the Division of Intramural Research (DIR) of the National Institute of Allergy and Infectious Diseases (NIAID). The DIR is the component of the National Institutes of Health (NIH) Intramural Program that conducts basic and clinical research studies in the areas of allergy, immunology, and infectious diseases. Over the years, the DIR scientists and support personnel have compiled an outstanding record of research that has given the DIR a worldwide reputation for scientific excellence.

This book describes the facilities of the DIR, the training opportunities for both clinical and laboratory research pathways, application information, overview of appointment mechanisms, and profiles of the laboratories and investigators that make up the DIR.

The information highlights the broad spectrum of research opportunities for applicants at various stages in their research careers. Training opportunities include programs for students and postdoctoral fellows, and accredited medical fellowship training programs in allergy/immunology and infectious diseases. The training environment in the DIR is enriched by opportunities to work side-by-side with renowned scientists and with colleagues from every part of the globe.

This book contains:

- General information about NIH, NIAID, and the NIAID DIR
- Information for application to clinical and laboratory research training programs
- Descriptions of the DIR laboratories and scientists
- Web addresses where you can get additional information.

We hope that you will find this book both informative and useful and that you will consider the DIR programs in your plans.

Who we are…

The National Institutes of Health

Since its beginnings in 1887, the National Institutes of Health (NIH) has grown from a one-person lab, housed in the attic of the Staten Island Marine Hospital in New York, to one of the world's largest and most influential medical research institutions. As an agency of the United States Public Health Service, the mission of the NIH is to uncover new knowledge that will lead to better health here and around the world. A few facts:

- NIH is composed of 27 Institutes, Centers, and Offices that support and conduct research in every biomedical discipline. The National Institute of Allergy and Infectious Diseases (NIAID) is the third largest of these, with over 1200 employees and hundreds of fellows.
- The main NIH campus in Bethesda, Maryland, includes 75 buildings on more than 300 acres. The NIAID Rocky Mountain Laboratories in Hamilton, Montana, occupy 32 newly renovated buildings located on 33 acres—with a breathtaking view of the Rockies.
- From a total of about $300 in 1887, the NIH budget has grown to more than $20.3 billion in 2001.

The National Institute of Allergy and Infectious Diseases

The NIAID provides the major support and direction for a large, nationwide cadre of scientists who conduct research aimed at understanding, treating, and preventing the many infectious, immunologic, and allergic diseases that afflict people throughout the world. This is accomplished through the NIAID's extramural grants programs, which provided over $1.2 billion to biomedical researchers in 2000, and through the NIAID's own staff of researchers in the Division of Intramural Research and the Vaccine Research Center, who receive about 11% of NIAID's budget. NIAID supported research includes:

- Studies of the basic mechanisms of normal immune function, including transplant immunology
- Dysfunction of the immune system resulting in autoimmunity, immunodeficiency, and allergy
- Studies of the basic biology, immunobiology, and molecular biology of pathogenic organisms.

The Division of Intramural Research

The DIR is one of six major components of NIAID. DIR comprises 15 Laboratories and 1 autonomous Section that conduct research, and 5 Branches that provide research technology and animal care services to DIR researchers. The DIR:

- Conducts research in virology, biochemistry, parasitology, epidemiology, mycology, molecular biology, immunology, immunopathology, and immunogenetics
- Supports clinical, patient-centered research in allergy, immunology, and infectious diseases at the Warren G. Magnuson Clinical Center on the NIH campus
- Employs more than 700 permanent staff and roughly 500 fellows
- Has laboratories located on the Bethesda, Maryland, campus of NIH, in Rockville, Maryland, a few minutes from campus, and at the Rocky Mountain Laboratories in Hamilton, Montana
- Provides a superb scientific setting for research and an unsurpassed training ground for new investigators.

The DIR Experience

Great Places

The DIR Laboratories are nestled in the park-like setting of the Bethesda, Maryland, main NIH campus, just outside of Washington, D.C. Our newly renovated laboratories in the Twinbrook buildings in Rockville, Maryland, are just a few minutes away. Plus, the DIR has world-class facilities in Montana at the Rocky Mountain Laboratories, featuring recently updated laboratories and a new biosafety level 3 lab building. Whether you prefer the excitement of the big city or the beauty of Montana's big sky, you'll feel at home in the DIR.

Exceptional People

This book features some of the best scientists in the world—the outstanding investigators of the DIR. Eight members of the current DIR staff have been elected to the National Academy of Sciences and many others have received prestigious awards for their contributions to science. The high quality of the DIR scientific staff is maintained through a rigorous review process. Approximately every 4 years, each DIR Laboratory and its investigators undergo a thorough review by a Board of Scientific Counselors, composed of academicians and industry representatives from outside of NIH. The Board evaluates the work of the researchers and makes recommendations for maintaining or improving the quality of the science. These recommendations form the basis for the DIR's ongoing efforts to conduct the highest quality research for the benefit of both public and individual health.

Outstanding Facilities

Just a few examples of the many amenities available to DIR employees and trainees:

- Research laboratories with state-of-the-art instrumentation and equipment
- The NIH Clinical Center, where scientific advances can quickly go from bench to bedside
- Common facilities for peptide synthesis, flow cytometry, confocal microscopy, and mass spectroscopy
- Small group and individual training in the use of specialized instrumentation
- In-house development of mouse, human, and microbial microarrays
- Transgenic and knock-out mice development and breeding
- Extensive in-house animal breeding and holding facilities
- Efficient computer networking and teleconferencing facilities, including satellite linkage to DIR-supported facilities in Africa.

Terrific Opportunities

The various NIH Institutes are home to more than 1,000 labs with scientists engaged in every area of biomedical research, providing an environment rich with expertise and opportunities for scientific exchange. Your scientific growth will be enhanced through:

- Inter-Institute Interest Groups, which join together senior scientists and fellows from different disciplines
- The NIH Fellows e-mail server, providing news and information of specific interest to fellows
- The Yellow Sheet, a daily listing of events, meetings, and lectures taking place at NIH
- The National Library of Medicine, featuring a huge catalog of on-line journals and numerous databases, plus outstanding libraries available in the Clinical Center and at the Rocky Mountain Laboratories
- Training programs for both laboratory and clinical researchers.

Laboratory Research Opportunities

Postdoctoral Training

If you're interested in postdoctoral laboratory research training, DIR has several options for you. Our programs consist of a minimum of 2–3 years of research in one of the DIR Labs, and both Ph.D. and M.D. candidates can apply. The available appointments differ slightly in their citizenship and postdoctoral experience requirements, but all have the same starting point—finding the best research fit for you. Start by reading the descriptions of the Laboratories and Investigators in this book and determining which DIR Lab or Investigator is conducting research in your area of interest. Then contact the Chief of the DIR Laboratory or the specific Investigator via telephone or e-mail to determine if an appropriate position is available. If you're asked to submit an application, follow the instructions below.

How to Apply

Wendy J. Fibison, Ph.D.

- Prepare a cover letter describing your background, research interests, career goals, and the special training or experience you're seeking.
- Include the date you can begin training, home address, home and office telephone numbers, a fax number, and e-mail address.
- Provide a copy of your curriculum vitae and bibliography. Representative publications are welcome.
- Mail the above information to the Lab Chief or Investigator you've contacted.
- If you wish your application to be more widely distributed in DIR, send the same information to Wendy Fibison, Ph.D., Associate Director for Special Emphasis Programs, Division of Intramural Research, Building 7, Room 300, MSC 0750, Bethesda, MD 20892; telephone 301-496-6400; fax 301-402-0077.

Appointment Mechanisms

If you're selected for an NIAID, DIR program, you may be appointed under any one of several mechanisms, depending on the availability of funding, type of research, and your qualifications. These appointment mechanisms include:

- **Postdoctoral Fellowship, including NIH Intramural Research Training Award (IRTA).** You must be a U.S. citizen or permanent resident with a doctoral degree and 5 or fewer years of postdoctoral experience.
- **Research Fellowship.** This appointment is for highly experienced postdoctoral scientists (generally more than 5 years postdoctoral experience) who seek further research training and additional professional development.
- **The NIH Visiting Program (VP).** This program offers scientists who are not U.S. citizens the opportunity to receive further training or to conduct research in their specialties. Appointments include:
  - **Postdoctoral Fellowship (VP).** You must have a doctoral degree or equivalent and 5 or fewer years of relevant postdoctoral experience.
  - **Research Fellowship (VP).** A Research Fellow (VP) must have a doctoral degree or equivalent and >5 years of relevant postdoctoral research experience.

Other Appointments

- **Adjunct Investigator.** This appointment is possible if you have outside funding and want to enhance your research capabilities in a DIR Laboratory. U.S. citizenship is not required.
- **Special Volunteer.** This appointment is suitable if you have funding from a foundation or private grant and wish to conduct research or be involved in patient care. You can provide patient care services

under the direct supervision and guidance of an NIH employee on the Clinical Center's senior active staff.

- **Guest Researcher.** This program allows you to use NIH facilities, equipment and resources for your research and training. However, you cannot provide services to the NIH.

Predoctoral Training for Students

- **Postbaccalaureate Intramural Research Training Award (IRTA).** If you're a recent college graduate, this program provides the opportunity to postpone your application to graduate or medical school so you can get an introduction to biomedical research that may encourage you to pursue a professional career in the field. To qualify, you must have graduated from a fully accredited U.S. college or university no more than 1 year prior to the activation date of the traineeship. Also, you must intend to apply to graduate or medical school in biomedical research within the next year.
- **Predoctoral IRTA.** This program is for students who have already been accepted into a doctoral program. To qualify, you must be accepted into or enrolled in a graduate, doctoral, or medical degree program and want to delay or interrupt your education for an interim research experience before entering school.
- **Technical IRTA.** This program is for applicants with a bachelor's or master's degree in a biomedical research field and fewer than 3 years of relevant postgraduate experience. It is designed to help you develop the advanced skills and techniques in basic and applied research necessary to be a highly trained research support professional.
- **Summer Internships.** Working in an NIAID laboratory for the summer can enhance your knowledge and understanding of the world of biomedical research and help you plan your academic goals. DIR offers 10–12 week summer internships for high school, college, graduate, and medical students. An online application for the following summer is available in early December of each year at http//www.training.nih.gov/student/internship/internship.asp. Application deadline is March 1.

Special Emphasis Programs

The DIR is committed to increasing the participation of women and underrepresented minorities in our research training programs. To that end, we sponsor events and programs designed to increase the visibility of DIR training opportunities among candidates who are African American, Hispanic/Latino, Native American, Alaskan Native, or Asian/Pacific Islander. The DIR Office of the Director sponsors research experiences for underrepresented minorities in our laboratories in Bethesda, Maryland and Hamilton, Montana, through a variety of programs. For example, we provide funding for up to ten predoctoral and five postdoctoral underrepresented minority trainees per year and sponsor NIH Undergraduate Scholarship Program and NIH Academy awardees. Information about the many training programs is available at http://www.training.nih.gov. Special seminars and events are planned for trainees in our programs to enhance their research experience in DIR.

We also participate in the Introduction to Biomedical Research Program (IBRP), which every year brings approximately 60 college students to NIH for a week of seminars and discussions with our scientists and exposure to the research environment. Application information for this program is available at http://www.niaid.nih.gov/OSPRT/IBRP.htm.

For more information about our programs for underrepresented minorities and women, please contact:

Dr. Wendy J. Fibison
Associate Director for Special Emphasis Programs
Building 7 Room 300
Mailstop Code 0750
Bethesda, MD 20892
Phone: 301-496-6400
Fax: 301-402-0077
E-mail: Wfibison@niaid.nih.gov

Clinical Training Opportunities

NIAID offers 3-year medical fellowships in ACGME-approved training programs in infectious diseases and allergy/immunology. These programs, described in detail below, aim to develop clinical and basic research skills in physicians who are already well grounded in clinical medicine and who seek to pursue a career in biomedical research. Applicants must have completed 3 years of residency training in an approved internal medicine or pediatrics residency training program in the United States or Canada before beginning a fellowship. Qualified individuals may apply for a student loan repayment program that currently repays up to $35,000 per year of eligible student debt.

The 3-year program comprises 1 year of clinical responsibilities and 2 years in research. All trainees spend 2 or 3 months of the first year caring for patients at NIH's Clinical Center, the nation's largest hospital devoted to clinical research. All NIAID patients participate in research protocols conducted by DIR investigators. Patients enter the Clinical Center with any of a variety of diseases, including:

- Autoimmune diseases, particularly vasculitides
- Genetic and acquired immunodeficiencies
- Disorders of neutrophil and monocyte function
- Severe, acute, and chronic viral infections, including herpes simplex, Epstein-Barr virus, and human immunodeficiency virus (HIV)
- Allergic diseases including asthma, anaphylaxis, and mast cell disorders
- Parasitic diseases
- Mycoses.

During the next 9 to 10 months of training, fellows join traditional consultation services and didactic rotations at the NIH and other medical institutions in the Baltimore-Washington, D.C., metropolitan areas. Following clinical training, fellows conduct research in any one of the DIR laboratories or other NIAID or NIH laboratories or programs.

Allergy/Immunology Training Program

Trainees who wish to become board-eligible in allergy and immunology are required to:

- Take rotations in allergy at various metropolitan area institutions
- Provide allergy and immunology consultation to the NIH Clinical Center
- Participate in allergy and immunology outpatient clinics
- Rotate through the ENT (ear, nose, and throat) and diagnostic laboratory
- Attend conferences
- Take American Board of Allergy and Immunology certification preparatory courses.

Subsequent immunology training in Clinical Laboratory Immunology (CLI) is available through the Immunology Service of the Clinical Pathology Department, Clinical Center. This 1-year training program leads to eligibility for a special certificate in CLI and is available to selected successful graduates of allergy and immunology training programs and graduates of specialty programs with a substantial immunology component.

Infectious Diseases Training Program

The infectious diseases training program accepts applications from residents in internal medicine who have completed training in the USA or Canada and who are not J1 visa holders. Applications are accepted from

August 1 of the second year before the fellowship begins up to the deadline date for the National Residency Matching Program, usually in May of the year prior to fellowship. Vacancies not filled through the Match are open for application after the Match. The first year of the training program is entirely clinical. Trainees who wish to become board-eligible in infectious diseases are required to:

- Spend 8 months of their first year on infectious diseases consultation services at the NIH Clinical Center, the Johns Hopkins Hospital, the George Washington University Medical Center, Georgetown University Medical Center, and Washington Hospital Center
- Learn hospital epidemiology and diagnostic microbiology
- Work at a sexually transmitted diseases clinic
- Attend journal club and case conferences regularly
- In the second year of infectious diseases training, continue attendance at case conferences and journal club
- Attend a continuity clinic for HIV-infected patients.

After completing the first year of clinical training, most trainees spend 2 or more years conducting research within NIAID DIR laboratories. Senior Investigators in these laboratories are involved in a variety of investigations in various aspects of allergy, clinical and basic immunology, and clinical and basic infectious diseases. Fellows work under direct supervision of a senior staff member. This arrangement allows for close daily contact, individual instruction, and continuity during the training period. This interaction between senior staff members and fellows also allows for the gradual maturation of fellows into independent investigators. Many fellows have established their own laboratories at medical schools and other research institutions following their NIAID training.

Current Research Projects

Current fellows in the training program are pursuing research projects that center around molecular biology as a tool for understanding virulence, host defense, inflammation, and allergy. Such projects include:

- Study of chemokines, lymphokines, fungal virulence, and drug resistance to fungi
- Cellular immune systems in inflammation and autoimmunity
- The function of cellular receptors for immunoglobulin and complement
- The basis of gastrointestinal and mucosal immunity
- Humoral and cellular immunoregulation and immunoregulatory defects
- Normal and abnormal biology of polymorphonuclear leukocytes, monocytes, and macrophages
- Pathophysiology of asthma and other allergic disorders
- Mast cell and basophil function
- Systemic mastocytosis
- Regulation of IgE and IgA synthesis
- The pathophysiology, molecular biology, and treatment of herpesvirus and other viral infections
- Aspects of the pathogenesis of HIV
- Immune-based therapies for HIV
- Pathophysiology of parasitic diseases.

Selected fellows may complete the research component of their training in any of the DIR laboratories listed in this book or work as part of a collaborative medical fellowship program with the Vaccine Research Center, or one of the extramural divisions of the Institute: the Division of Acquired Immunodeficiency Syndrome (DAIDS), the Division of Allergy, Immunology, and Transplantation (DAIT); the Division of Microbiology and Infectious Diseases (DMID); or, by special arrangement, with other NIH institutes.

How and When to Apply

Applicants should follow the instructions contained in the application package available from the Training Program directors listed below. Included in the application package is a request to submit two copies of the following:

- A cover letter describing the program to which you wish to apply, your background, research interests, career goals, and the special training or experience you are seeking at NIH. Also indicate the year for which you are applying to begin your training, your home address, home and office telephone numbers, and facsimile number and e-mail address, if you have them.
- A copy of your *curriculum vitae* and bibliography. Representative publications are welcome.
- Copies of your undergraduate and medical school/graduate school transcripts.
- Three letters of recommendation emphasizing your potential for clinical research and/or laboratory research training, and your scholastic aptitude from program directors and/or laboratory chiefs at your current training institution. For the Combined Clinical and Research Pathway, two of the three letters must be from medical faculty and must address your relevant clinical skills.


Dean D. Metcalfe, M.D.

Allergy/Immunology Training Program. Request an application packet from or send information to Dean D. Metcalfe, M.D., Building 10, Room 11C205, 10 Center Drive, MSC 1881, Bethesda, MD 20892-1881. For further information, contact Dr. Metcalfe's office: telephone 301-496-2165; fax 301-480-8384; or e-mail to dean_metcalfe@nih.gov. Applications can also be requested by calling (301) 496-3591.


John E. Bennett, M.D.

Infectious Diseases Training Program. Request an application from or send information to John E. Bennett, M.D., Building 10, Room 11C304, 10 Center Drive, MSC 1882, Bethesda, MD 20892-1882. For further information, contact Dr. Bennett's office: telephone 301-496-3461; fax 301-480-0050; or e-mail jb46y@nih.gov.

The Infectious Diseases Training Program participates in the National Infectious Diseases Matching Program. Programs and applicants to the match submit final selections in the third week of May in the year prior to starting fellowship. Interviews are held during the 6 months prior to the match.

For the Allergy/Immunology training program, applications may be considered at any time that an opening is available in a fellowship class, although the ideal time to apply is from November to January, beginning 2 years before the actual fellowship. For example, the best time to apply for a July 1998 fellowship was November 1996 to January 1997.

The Selection Process

Candidates are selected for interview based on their clinical and/or research credentials and research interests. Interview visits to the NIH campus are designed to introduce potential trainees to NIH preceptors and to provide the candidate with the opportunity to explore in detail the nature of the research he or she might conduct.

A candidate selected from the interviewees is offered a position that may be based on a number of funding mechanisms, depending on availability of funding, the type of research to be conducted, and the qualifications of the candidate.

Individuals with future appointment commitments may be eligible for the NIH Loan Repayment Program. This is a competitive process with several categories of loans for which the fellow may apply. These are described on the next page. A limited number of applicants will be selected.

Loan Repayment Programs

General Loan Repayment Programs

The NIH General Loan Repayment Program (LRP), authorized by Congress in 1993, was established to attract highly qualified professionals, particularly physicians, to conduct research at NIH. Unlike previously authorized LRPs that targeted specific areas or types of research, such as AIDS or clinical research, this program supports "research generally" in a variety of scientific disciplines. The General LRP may repay up to a maximum of $35,000 per year toward participants' outstanding eligible education loans. In return, participants must sign a contract agreeing to conduct qualified research activities as NIH employees for a maximum of 3 years. Additional information can be found at http://lrp.info.nih.gov/. The NIH General Research LRP for Accreditation Council for Graduate Medical Education (ACGME) Fellows is a pilot initiative of $5,000 per year in loan repayments, currently available for fellows employed by the NIH in subspecialty and residency training programs accredited by the ACGME. Qualifying fellows must hold a 3-year appointment at NIH beginning July 2000, 2001 or 2002.

AIDS Loan Repayment Program

NIAID's AIDS research encompasses work on the etiological agent, pathogenesis, therapeutics, vaccine development, and epidemiology and natural history of HIV infection. AIDS research will continue to require dedicated, well-trained basic and clinical scientists into the foreseeable future. The educational debt repayment program was instituted to permit qualified postdoctoral physicians and scientists to enter this expanding area of research. For each year of full service as NIH employees primarily engaged in AIDS research in any one of the Institutes, physicians and scientists may have up to $20,000 of their qualified educational loans repaid. In exchange for loan repayment benefits, researchers must agree to participate in AIDS research for a minimum of 2 years. Continuation contracts for additional years may be entered subsequently. To be eligible, you must be a citizen of the United States or a permanent resident, hold a Ph.D., M.D., D.O., D.D.S., D.M.D., D.V.M., A.D.N./B.S.N., or equivalent degree, and have qualifying educational debt in excess of 20 percent of your annual NIH basic pay or stipend at the effective date of program participation. Additional information regarding this program can be found at http://www.niaid.nih.gov/dir/training/loan.htm or at http://lrp.info.nih.gov/.

Clinical Research Loan Repayment

The NIH Clinical Research Loan Repayment Program (CR-LRP) is designed to recruit highly qualified physicians, nurses, and scientists from disadvantaged backgrounds to serve as clinical researchers. Eligibility requirements for the CR-LRP are the same as those for the AIDS Loan Repayment Program above, with two additional criteria: 1) you must be from a disadvantaged background, and 2) you must be awarded clinical privileges by the Clinical Center Medical Board or other credentialing board upon NIH employment. An individual from a disadvantaged background is defined as one who comes from: 1) an environment that inhibited (but did not prevent) him or her from obtaining the knowledge, skill, and ability required to enroll in and graduate from a health professions school, or 2) a family with an income below a level based on low-income thresholds. This level considers family size and Bureau of the Census statistics, with annual adjustments for changes in the Consumer Price Index. The DHHS Secretary adjusts this level for use in all health professions programs and publishes this information periodically in the Federal Register. Additional information about eligibility criteria and application procedures can be found at http://lrp.info.nih.gov/aib/aib2.htm#General.

For additional information and application forms, visit http://lrp.info.nih.gov/ or contact us at:
NIH Loan Repayment Program, 2 Center Drive, MSC 0230, Bethesda, MD 20892-0230,
Phone: 800-528-7689 or 301-402-5666, FAX: 301-402-8098.

Tenure and Tenure Track in NIAID

The primary purpose of an NIH fellowship or clinical associateship is to provide time-limited research training and career development opportunities to postdoctoral scientists. At the end of the training period, the majority of fellows will leave NIH to pursue careers at institutions in the United States or abroad; however, some intramural fellows may be selected to compete for permanent positions as tenured independent investigators. Tenure at NIH consists of a permanent position and a long-term commitment of salary, personnel, and the research resources needed to conduct an independent research program within the scope of the Institutes' mission.

Intramural scientists obtain tenure in one of two ways: (1) the scientist is recruited from outside NIH for a tenured position after having compiled an extensive research record at another institution, or (2) the scientist enters a tenure-track position after extended research experience at NIH or elsewhere. This experience should be sufficiently extensive to allow thorough evaluation of an individual's potential as a tenure-track scientist. In both cases, the position is recommended by the Board of Scientific Counselors and Laboratory Chief and approved by the Director, DIR, and Director, NIAID.

Candidates for both tenure and tenure-track positions are selected by a search committee and approved by the Deputy Director of Intramural Research, NIH. Tenure-track candidates are given 6 years to establish themselves as independent scientists before being evaluated for tenure. The Board of Scientific Counselors reviews the candidate's performance and qualifications for tenure at the midpoint of the tenure-track clock and decides whether the candidate should be continued in tenure-track, dropped from track, or advanced for tenure decision.

Regardless of the route by which an individual gains eligibility for a tenured position, the tenure candidate will come before the NIAID Promotions and Tenure Committee, which advises the Director, DIR, on whether to grant tenure. If the committee recommends tenure and the Director, DIR, concurs, the request is forwarded to the NIH Central Tenure Committee, which is chaired by the Deputy Director for Intramural Research, NIH, for approval.

Timeline for Nontenured Staff at NIH

The initial fellowship appointment is for a period of 2-3 years. This may be renewed at the request of the host laboratory, if it is mutually beneficial to do so. It is the usual policy of NIH that postdoctoral trainees should not remain at NIH for more than 5 years. The overall limitation is not more than 8 years, regardless of appointment mechanism, unless the postdoctoral trainee is approved for tenure-track or a permanent appointment.

DIR Laboratories

Laboratory of Allergic Diseases

Dean D. Metcalfe, M.D., Chief

Http://www.niaid.nih.gov/dir/labs/lad.htm

Phone: (301) 496-2165

Laboratory Sections and Units

Office of the Chief

Dean D. Metcalfe, M.D.

Receptor Cell Biology Section

John Coligan, Ph.D., Head

Mast Cell Biology Section

Dean D. Metcalfe, M.D., Head

Molecular Signal Transduction Section

Kirk Druey, M.D., Head (Acting)

Eosinophil Biology Section

Andrea Keane-Myers, Ph.D., Head (Acting)

Clinical Allergy and Immunology Unit

Calman Prussin, M.D., Head

Research Activities

The Laboratory of Allergic Diseases (LAD) conducts basic and clinical research on immunologic diseases, with an emphasis on disorders of immediate hypersensitivity, which include the spectrum of classic allergic diseases. LAD is composed of an interactive group of Ph.D. and M.D. researchers, research nurses, and technicians who work in contemporary laboratories. Personnel are engaged in studies to elucidate the events in allergic inflammatory reactions. Basic research efforts are directed at studying the growth and differentiation of mast cells, inflammatory cell surface receptors, signal transduction pathways in inflammation, and finally, the biologic expression in tissues of effector cell activation. Clinical research is directed toward understanding the pathogenesis of allergic inflammation and the role of T lymphocytes and their cytokines in this process. Efforts are then undertaken to translate basic and clinical research findings into novel immunomodulatory and anti-inflammatory approaches to the treatment of allergic and immunologic disorders, including asthma and systemic mast cell diseases.

Dean D. Metcalfe, M.D.

Chief, Laboratory of Allergic Diseases
Head, Mast Cell Biology Section, LAD

http://www.niaid.nih.gov/dir/labs/lad.htm
E-mail: dmetcalfe@nih.gov

Dr. Metcalfe received his undergraduate degree from Northern Arizona University, his M.D. from the University of Tennessee, and his training in internal medicine at the University of Michigan. Dr. Metcalfe was a Fellow in allergy and immunology at NIH and a Fellow in rheumatology and immunology at the Robert Brigham Hospital and the Harvard Medical School. In 1979, he returned to NIH as a Senior Clinical Investigator. In 1995, Dr. Metcalfe was appointed the Chief of the Laboratory of Allergic Diseases, NIAID. Dr. Metcalfe is an author on over 325 scientific publications on mast cell biology, food allergy, mastocytosis, and asthma. He is a past Chairman of the American Board of Allergy and Immunology, and has been elected to membership in the American Association of Physicians.

Description of Research Program

The mast cell is the focus of the MCBS research effort, because this cell plays a central role in the induction of allergic inflammation. An integrated program investigating mast cell biology includes studies into the growth and differentiation of mast cells, mast cell signal transduction, and the products generated by mast cells that lead to disease. Research efforts have contributed to understanding the identification of mutations in the receptor for stem cell factor; in signaling through high affinity IgE and IgG receptors; and the demonstration of regulation of tissue mast cell number through programmed cell death. Future efforts will be directed at further characterization of mast cell precursors: characterization of signaling pathways in human mast cells; examination of the role of programmed cell death in regulating mast cell populations; characterization of new and novel mast cell mediators; and application of this information to the diagnosis and treatment of asthma and other allergic and immunologic diseases.

Major Areas of Research

- Mast cell growth and differentiation
- FcεRI and Fcγ mediated signal transduction in mast cells
- Characterization of c-kit mutations associated with systemic mastocytosis

Selected Recent Publications

Kirshenbaum, A.S., Goff, J.P, Semere, T., Scott, L.M. and Metcalfe, D.D. Demonstration that human mast cells arise from a bipotential progenitor cell population that is CD34+, c-kit+, and expresses aminopeptidase N (CD13). *Blood* 94:2333-2342, 1999.

Okayama, Y., Kirshenbaum, A.S., and Metcalfe, D.D. Expression of a functional high affinity IgG receptor (FcγRI) on human mast cells: Up-regulation by IFN-γ. *J. Immunol*. 164:4332-4339, 2000.

Akin, C., Schwartz, L.B., Kitoh, J., Obayaski, A., Worobec, A.S., Scott, L.M., and Metcalfe, D.D. Soluble stem cell factor receptor (CD117) and IL-2 receptor (CD25) levels in the serum of patients with mastocytosis: Correlation with clinical severity and bone marrow pathology. *Blood* 96: 1267-1273, 2000.

Daley, T, Metcalfe, D.D., and Akin, C. Association of the Q576R polymorphism in the interleukin-4 receptor alpha chain with indolent mastocytosis limited to the skin. *Blood*. 98:1195-1199, 2001.

Hagaman, D.D., Okayama, Y., Gilfillan, A.M., Prussin, C., and Metcalfe, D.D. Secretion of interleukin-1 receptor antagonist from human mast cells following IgE mediated activation and following segmental antigen challenge. *Am. J. Resp. Cell. Mol. Biol*. 25:685-691, 2001.

John E. Coligan, Ph.D.

Head, Receptor Cell Biology Section, LAD

Web address: http://www.niaid.nih.gov/dir/labs/lad/recept.htm

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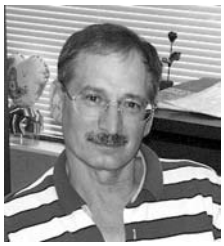

Dr. Coligan obtained his M.S. and Ph.D. degrees in microbiology from Indiana University. After postdoctoral studies in tumor immunology at the Beckman Research Institute City of Hope, he spent two years at the Rockefeller University as an Assistant Professor. He joined the Laboratory of Immunogenetics, NIAID, in 1977, becoming a Section Head shortly thereafter. In 1986, he founded the Biological Resources Branch and in 1994, the Laboratory of Molecular Structure. He has been with the Laboratory of Allergic Diseases since 1999. He is the co-author of over 300 publications.

Description of Research Program

Studies focus on the molecular mechanisms that determine natural killer (NK) cell function. These include: 1) determination of how members of the CD94/NKG2 family of receptors for HLA-E class I molecules effect target recognition and how post recognition signals that dictate lysis or inhibition of lysis are transmitted; 2) determination of the factors and processes that regulate tissue-specific and clonal expression of CD94/NKG2 family genes; 3) understanding the relationship between intracellular trafficking and function of the CD94/NKG2 family of receptors; 4) the use of gene microarray analyses to elucidate expression of genes and complexes of genes that are important for NK cell function; 5) understanding the role of adhesion molecules, particularly integrins, in lymphocyte development, trafficking and function.

Major Areas of Research

- Transcriptional regulation of CD94/NKG2 gene expression
- Use of gene microarray analyses to determine NK gene expression in response to external stimuli
- Analysis of the molecular interactions between CD94/NKG2 receptors and their ligand(s)
- Determination of how CD94/NKG2 family members transmit inhibitory and activating signals to NK cells
- Visualization of NK receptor intercellular synapses with target cell ligands
- Selective thymus knockout of β_1, $\alpha 4$, and $\alpha 6$ integrin gene expression in order to determine the role that b_1 integrins play in T-cell development, trafficking and function

Selected Recent Publications

Magner, W.J., A.C. Chang, J. Owens, M-JP Hong, A. Brooks and J.E. Coligan. Aberrant development of thymocytes in mice lacking laminin-2. *Developmental Immunol.* 7: 179-193, 2000.

Brooks, A.G., F. Borrego, P.E. Posch, A. Patamawenu, C.J. Scorzelli, M. Ulbrecht, E.H. Weiss, and J.E. Coligan. Specific recognition of HLA-E but not classical HLA class I molecules by soluble CD94/NKG2A and NK cells. *J. Immunol.* 162: 305-313, 1999.

Borrego, F., M. Ulbrecht, E.H. Weiss, J.E. Coligan, and A.G. Brooks. Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *J. Exp. Med.*187: 813-818, 1998.

Posch, P.E., F. Borrego, A.G. Brooks, and J.E. Coligan. HLA-E is the ligand for the natural killer cell CD94/NKG2 receptors. *J. Biomed. Sci.* 5: 321-331, 1998.

Halvorson, M.J., W. Magner, and J.E. Coligan. $\alpha 4$ and $\alpha 5$ integrins co-stimulate the CD3-dependent proliferation of fetal thymocytes. *Cell Immunol.* 189: 1-9, 1998.

Kirk M. Druey, M.D.

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Dr. Druey received his medical degree from Rush Medical College in Chicago, Illinois, in 1987, and completed a medical residency in internal medicine at the New York Hospital/Cornell Medical Center in 1990. He entered the Public Health Service (PHS) Commissioned Corps in NIAID in 1991. After completing a clinical year under the Office of the Clinical Director, he completed an additional 5 years of postdoctoral training in the B-Cell Molecular Immunology Section, Laboratory of Immunoregulation, NIAID, under the direction of Dr. John Kehrl. He was named Investigator (tenure-track) in LAD in 1997.

Description of Research Program

Our section studies signal transduction by heterotrimeric GTP binding proteins (G proteins) and the role of G-protein-mediated signaling in the initiation and propagation of allergic inflammatory processes. In asthma, for example, G-protein-coupled receptors (GPCRs) control airway smooth muscle contraction (adrenergic and muscarinic), inflammatory mediator effects (leukotriene, platelet activating factor), and the influx and trafficking of inflammatory cells into the lung (chemokine).

Current work involves characterization of regulator of G-protein signaling (RGS) proteins, which enhance the intrinsic GTPase activity of heterotrimeric G protein alpha subunits and are negative regulators of GPCR signal transduction. We are studying RGS4, expressed in mast cells; RGS16, expressed in T lymphocytes; and RGS13, an uncharacterized RGS protein expressed in lung. We would like to determine which proteins regulate specific G-protein-coupled pathways involved in immune and inflammatory processes.

Thus, a principal focus of our research is to define targets of these RGS proteins. We have characterized some of their biochemical properties *in vitro.* Utilizing enzymatic assays with recombinant proteins as well as expression in cultured cell lines, we determined that specificity of RGS proteins may be regulated transcriptionally as well as by post-translational modifications such as palmitoylation, phosphorylation, and endoproteolytic processing. In order to investigate the physiological targets of these RGS proteins in the whole organism, we are currently generating mice deficient in RGS4 or RGS13. Studies of these mice should allow us to ascertain which GPCR pathways might be dysregulated in a variety of pathologic conditions.

Major Areas of Research

- G-protein signal transduction
- RGS proteins
- Eosinophil signal transduction

Selected Recent Publications

Beadling, C., K. M. Druey, G. Richter, J. H. Kehrl, and K. M. Smith. Regulators of G protein signaling exhibit distinct patterns of gene expression and target G protein specificity in human lymphocytes. *J. Immunol.* 162: 2677-82, 1999.

Druey, K.M., O. Yazar-Ugur, J. M. Caron, C. K. Chen, P. S. Backlund, and T.L.Z. Jones. Amino terminal cysteine residues of RGS16 are required for palmitoylation and modulation of Gi and Gq mediated signaling. *J. Biol. Chem.* 274: 18836-12, 1999.

Cavalli, A., K. M. Druey, and G. Milligan. The regulator of G$_{\sim}$protein signaling RGS4 selectively enhances α_{2A} adrenoceptor stimulation of the GTPase activity of $G_{o1\alpha}$ and $G_{i2\alpha}$. *J. Biol. Chem.* 275: 23693-23699, 2000.

Sullivan, B.M., K. J. Harrison-Lavoie, V. Marshansky, H. Y. Lin, J. H. Kehrl, D. A. Ausiello, D. Brown, and K.M. Druey. RGS4 and RGS2 bind coatomer and inhibit COPI association with Golgi membranes and intracellular transport. *Mol. Biol. Cell* 11: 3155-3168, 2000.

Andrea Keane-Myers, Ph.D.

Acting Section Head, Eosinophil Biology Section, LAD

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Dr. Keane-Myers received her bachelor of science in biology from the University of Richmond, Virginia. She received her Ph.D. in immunology and molecular microbiology from Johns Hopkins University in 1995. She then completed a postdoctoral fellowship in pulmonary medicine at Johns Hopkins Medical Institutions. She completed a second postdoctoral fellowship and became an Instructor in molecular immunology at the Schepens Eye Research Institute/ Harvard Medical School. She was named Investigator (tenure-track) in the Eosinophil Biology Section LAD/NIAID in the fall of 2000.

Description of Research Program

Studies focus on the use of animal models to investigate the role of inflammatory cells in the immediate phase of the allergic inflammatory cascade. In our primary mouse model, we challenge animals via aerosolization of allergens and assess lung function and inflammation. This model allows us to not only determine the inflammatory response in the lung (or lack thereof), but also how such inflammation affects the airway response. In a secondary model, we use topical allergen challenge to induce allergic conjunctivitis. This model allows us to easily observe mast cell degranulation and mucous production in a local inflammatory response, and allows for topical treatment regimens.

We are currently investigating the relative impact of various inflammatory cells (mast cells, T cells, basophils, eosinophils, and neutrophils) in allergen-induced cytokine production immediately after allergen challenge. We are using a number of transgenic and knockout mice to assess the role(s) each of these cells are playing in the allergic inflammatory cascade. We are also developing transgenic mice that will have enhanced lung-specific cellular populations, as we believe these will be better models of human allergic asthma. Finally, we are developing novel ways to track antigen-specific cellular infiltration into the lungs following allergen challenge in order to assess the role that these cells have in both the immediate and chronic allergic responses.

Major Areas of Research

- Immediate/early events in allergic asthma including IL-4 production
- Infiltration of mast cells, T cells and eosinophils into sites of allergic inflammation
- Biologic expression in tissues of effector cell activation

Selected Recent Publications

Keane-Myers, A.M., F. D. Finkleman, W. C. Gause, and M. Wills-Karp. Development of murine allergic asthma is dependent upon B7-2 not B7-1 costimulation. *J. Immunol.* 160: 1036-1043, 1998.

Keane-Myers, A.M., M. Wysocka, G. Trinchieri, M. Wills-Karp. Resistance to antigen-induced airway hyperresponsiveness requires endogenous production of interleukin-12. *J. Immunol.* 161: 919-926, 1998.

Keane-Myers, A. M., G. Liu, D. Miyazaki, I. Dekaris, S. Ono, and M. R. Dana. Treatment with IL-1 receptor antagonist prevents allergic eye disease. *Invest. Opthalmol. Vis. Sci.* 40: 3041-6, 1999.

Casolaro V., A. M. Keane-Myers, S. L. Swendeman, C. Steindler, F. Zhong, M. Sheffery, S. N. Georas, and S. J. Ono. Identification and characterization of a critical CP2-binding element in the human interleukin-4 promoter. *J. Biol. Chem.* 24; 275(47): 36605-11, 2000.

Calman Prussin, M.D.

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Dr. Prussin received his bachelor of arts in chemistry from the University of California San Diego in 1980. He received his medical degree in 1984 and internal medicine training from 1984 through 1988, both from the University of Southern California Medical School. From 1989 through 1991, he did postdoctoral training within the Laboratory of Cellular and Molecular Immunology before he began clinical allergy and immunology training within the Allergic Diseases Section, Laboratory of Clinical Investigation (1991 to 1995). Dr. Prussin was promoted to his current position as Head of the Clinical Allergy and Immunology Unit in 1997. He is board certified in both internal medicine and allergy and immunology.

Description of Research Program

Current studies are focused on understanding the role of immunological tolerance in asthma with the goal of applying this knowledge to asthma therapeutics. Using highly sensitive assays of allergen-specific cytokine production, we have demonstrated that non-allergic controls exhibit T-cell non-responsiveness to allergen, whereas allergic asthmatics clearly demonstrate a Th2 response. These results suggest that the therapeutic generation of T-cell tolerance to allergens may be a desirable clinical goal. Current research is focused on characterizing allergen specific regulatory T cells to determine their role in the maintenance of tolerance to aeroallergens.

Current clinical protocols are aimed at examining the role of tolerance in immunotherapy. Our hypothesis is that allergen immunotherapy acts by tolerizing Th2 cells, rather than by causing immune deviation of Th2 cells into Th0 or Th1 cells. Our goal is to translate this understanding into more effective immunomodulatory strategies.

Using similar techniques, we have examined the role of basophils in allergic disease and asthma and have shown that following allergen activation, basophils comprise the vast majority of IL-4 producing cells relative to T cells. Additionally, we have shown that chemokines potentiate allergen dependent basophil IL-4 production and cause a 40-fold shift in the dose response to allergen.

Major Areas of Research

- Understanding the mechanism of immunological tolerance to aeroallergens
- Characterization of allergen specific regulatory T cells
- Defining the contribution of basophils to asthmatic inflammation

Selected Recent Publications

Stobie L., S. Gurunathan, C. Prussin, D. L. Sacks, N. Glaichenhaus, C. Y. Wu and R. A. Seder. The role of antigen and Il-12 in sustaining Th1 memory cells *in vivo*: IL-12 is required to maintain memory/effector Th1 cells sufficient to mediate protection to an infectious parasite challenge. *Proc. Natl. Acad. Sci. USA* 97: 8427-32, 2000.

Devouassoux G., D. D. Metcalfe, C. Prussin. Eotaxin potentiates antigen dependent basophil IL-4 production. *J. Immunol.* 163: 2877-2282, 1999.

Devouassoux G., B. Foster, L. M. Scott, D. D. Metcalfe and C. Prussin. Frequency and characterization of antigen specific IL-4 and Il-13 producing basophils and T cells in peripheral blood of health and asthmatic subjects. *J. Allergy Clin. Immunol*. 104: 811-819, 1999.

Gurunathan S., C. Prussin, D. L. Sacks and R.A. Seder. Vaccine requirements for sustained cellular immunity to an intracellular parasitic infection. *Nature Med.* 4: 1409-1415, 1998.

Laboratory of Cellular and Molecular Immunology

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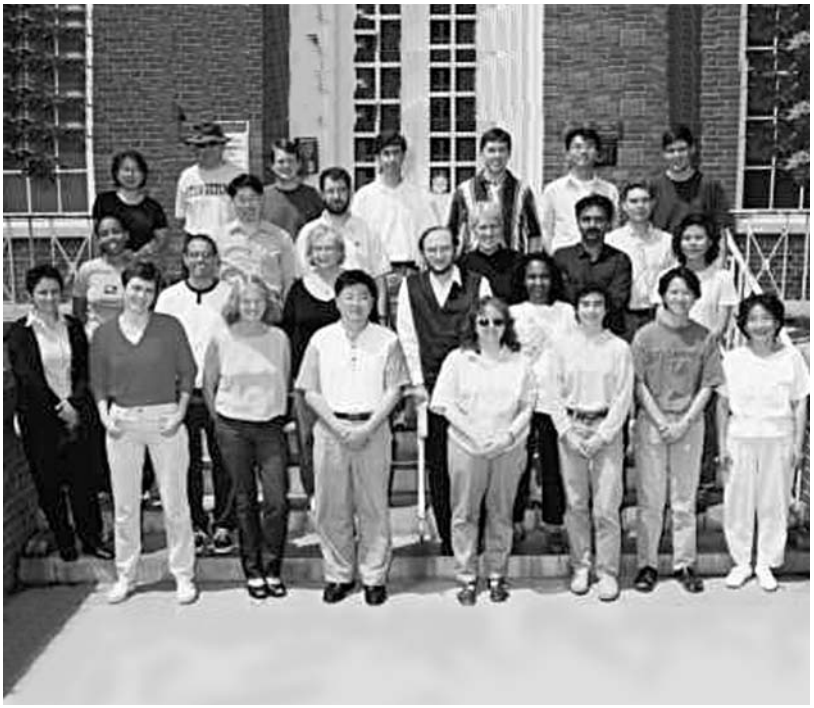
Ronald H. Schwartz, M.D., Ph.D., Head

T-Cell Development Section

B. J. Fowlkes, Ph.D., Head

T-Cell Tolerance and Memory Section

Polly Matzinger, Ph.D., Head



Research Activities

The Laboratory of Cellular and Molecular Immunology (LCMI) studies the development, activation, tolerance, memory, differentiation, and death of thymus-derived (T) lymphocytes. Research projects currently focus on lineage commitment in the thymus, including the role of notch proteins, the role of danger and costimulatory signals in initiating and controlling the class of an immune response, molecular and cellular aspects of T-cell anergy, the cloning of genes from thymic epithelial cells, and dissection of *in vivo* models of $CD4^+$ T-cell tolerance. Educational experiences are offered in immunology through tutorials and weekly journal clubs in addition to research training in techniques such as flow cytometry, gene cloning, and T-cell cloning. All research is carried out using mouse models.

Ronald H. Schwartz, M.D., Ph.D.

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Head, T-cell Activation Section

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Dr. Schwartz was a chemistry major at Cornell University and graduated *summa cum laude* in 1965. He then attended Harvard Medical School from which he graduated *cum laude* in 1970. He completed a Ph.D. at the Institute of Microbiology in 1973 on the interaction of synthetic polypeptides with macrophage RNA. He came to NIH in 1972 and was first appointed as a Staff Scientist in the United States Public Health Service working on separation procedures for cells in the immune system. In 1974, Dr. Schwartz was appointed as a Research Associate in the Laboratory of Immunology where he began his work on immune response gene control of T-cell proliferative responses in the mouse. He became a tenured Senior Investigator in NIAID in 1976 and subsequently made a number of seminal observations on the nature of the processed antigen recognized by T cells. In 1986, he became the Chief of the Laboratory of Cellular and Molecular Immunology and subsequently discovered the tolerance phenomenon of T-cell clonal anergy. More recently his laboratory discovered the $CD4^+$ NK 1.1T cell, and he is currently working on *in vivo* models of T-cell tolerance and a molecular understanding of T-cell clonal anergy.

Description of Research Program

The research program is focused in two areas. The first is the cellular and molecular aspects of T-cell anergy. Our group did the pioneering work in describing this phenomenon in the activation of $CD4^+$ T-cell clones and is now attempting to understand them *in vivo* and at a molecular level. Molecular projects focus on the nature of the *cis*-dominant negative regulation of IL-2 gene transcription in anergy and identification of genes responsible for inducing and maintaining the anergic state. Cellular projects are examining the mechanism of anergy *in vivo* using two model systems. One involves administration of the superantigen SEA to a T-cell receptor transgenic mouse, and the other involves the transfer of naive $CD4^+$ T cells from that mouse into another transgenic animal expressing the antigen for which the T cell is specific. The second area is the characterization of subsets of cells in the thymus. Our group was the first to describe the $CD4^+$ $NK1.1^+$ T cell subset in the mouse. Current projects are focused on cloning genes specifically expressed in thymic epithelial cells and studying their function by gene targeting and blocking with antibodies in fetal thymic organ cultures. Students receive training in both cellular and molecular immunology.

Major Areas of Research

- T-cell anergy, tolerance, and activation
- Thymic stromal cell genes

Selected Recent Publications

Miller, C., Ragheb, J.A., and Schwartz R.H. Anergy and cytokine-mediated suppression as distinct superantigen-induced tolerance mechanisms *in vivo. J. Exp. Med.* 190:53-64, 1999.

Kim, M.G., Flomerfelt, F.A., Lee, K.-N., Chen, C., and Schwartz R.H. A putative12 transmembrane domain co-transporter from thymic stromal cells that participates in early thymocyte development. *J. Immunol.* 164: 3185-3192, 2000.

Kim, M.G. Lee, G, Lee, S.-K., Lolkema, M., Yim, J., Hong, S.H., and Schwartz, R.H. Epithelial cell-specific laminin 5 is required for survival of early thymocytes. *J. Immunol.* 165: 192-201, 2000.

Flomerfelt, F.A., Kim, M.G., and Schwartz, R.H. Spatial, a gene expressed in thymic stromal cells, depends on 3-dimensional thymus organization for its expression. *Genes and Immunity* 1: 391-401, 2000.

Chiodetti, L., Barber, D.L., and Schwartz, R.H. Biallelic expression of the IL-2 locus under optimal stimulation conditions. *Eur. J. Immunol.* 30: 2157-2163, 2000.

Tanchot, C., Barber, D.L., Chiodetti, L., and Schwartz, R.H. Adaptive tolerance of $CD4^+$ T cells in vivo: Multiple thresholds in response to a constant level of antigen presentation. *J. Immunol.* 167: 2030-9, 2001.

B.J. Fowlkes, Ph.D.

Head, T-Cell Development Section, LCMI

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After receiving a M.S. degree from the Medical College of Virginia for studies in *Drosophila* genetics, Dr. Fowlkes conducted research on cancer at the NCI and immunology at the NIAID before receiving her Ph.D. degree for studies on thymocyte differentiation at George Washington University. She joined the Laboratory of Cellular and Molecular Immunology in 1987, was tenured as a Senior Investigator in 1990, and was made Head of the T-Cell Development Section in 1992. Since 1999, she has served as Adjunct Professor of Genetics and of Microbiology/ Immunology at George Washington University. She serves on numerous editorial and scientific advisory boards and is a recipient of a Roche Basic Science Award, NIH Merit Award, and the American Association of Immunology Investigator Award for outstanding contributions to Immunology.

Description of Research Program

The Section conducts research in developmental immunology. Since its initial work isolating and characterizing the earliest precursor T cells in the adult thymus, the laboratory has investigated precursor/product relationships and described the stages and kinetics of T-cell development. In the first studies demonstrating that the T-cell antigen receptor (TCR) associated with CD3 is expressed on precursor thymocytes, two novel populations of mature thymocytes bearing TCR$\gamma\delta$ or TCR$\alpha\beta$, but lacking surface expression of CD4 and CD8, were identified. This ability to analyze TCR expression and the TCR repertoire gave new insight into thymic selection, the mechanisms that either promote development or prevent the maturation of T cells expressing useless or self-reactive receptors. Analyses of the interactions of specific TCR, the coreceptors (CD4/8), and other receptors on thymocytes with superantigen and MHC ligands on thymic stromal cells, provided evidence that TCR affinity determines positive *vs.* negative selection. This work also revealed that the thymic epithelium is able to induce a non-deletional form of tolerance (clonal anergy) for preventing self-reactivity. The current projects of the unit are focused on identifying the intra- and extra-cellular signals controlling cell fate decisions in thymocytes. These include signals affecting differentiation, survival, and death, but also those involved in the development of lineages. Results from transgenic and gene-targeted mutant mice in which TCR signal transduction or coreceptor expression is altered suggest that the quantity of TCR signal influences CD4 vs. CD8 T-lineage commitment. The transmembrane receptor, Notch, also plays a role in CD4 vs. CD8, as well as the $\gamma\delta$ *vs.* $\alpha\beta$, T-lineage decisions. The goal now is to understand how TCR signals are integrated with other developmental cues to specify T-cell fate.

Major Areas of Research

- T-cell development
- Thymic selection
- T-lineage commitment

Selected Recent Publications

Matechak, E.O., Killeen, N., Hedrick, S.M., and Fowlkes, B.J. MHC class II-specific T cells can develop in the CD8 lineage when CD4 is absent. *Immunity* 4: 337-347, 1996.

Robey, E.A., and Fowlkes, B.J. The $\alpha\beta$ versus $\gamma\delta$ T cell lineage choice. *Curr. Opin. Immunol.* 10: 181-187, 1998.

Terrence, K., Pavlovich, C.P., Matechak, E.O., and Fowlkes, B.J. Premature Expression of transgenic TCR$\alpha\beta$ suppresses endogenous TCRγ/δ rearrangement but permits development of the $\gamma\delta$ lineage T cells. *J. Exp. Med.* 197: 537-548, 2000.

Polly Matzinger, Ph.D.

Head, T-Cell Tolerance and Memory Section, LCMI

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Dr. Matzinger obtained her Ph.D. in biology from the University of California at San Diego for the study of T-cell tolerance. Following 4 years of postdoctoral research at Cambridge University, England, and 6 years at the Basel Institute of Immunology, Switzerland, she joined the Laboratory of Cellular and Molecular Immunology in 1989 and became a Section Head in 1995. Dr. Matzinger's research focuses on the fundamental principles by which the immune system operates. Dr. Matzinger serves on numerous editorial boards, is a member of the advisory board for the Council for the Advancement of Science Writing, is an award-winning filmmaker, and writes and speaks to lay as well as scientific audiences.

Description of Research Program

The T-Cell Tolerance and Memory Section is engaged in elucidating the answers to three fundamental questions in immunology: (1) What turns an immune response on and off? (2) How does the immune system remember its past encounters? (3) What regulates the effector class of an immune response?

The Section is currently working on the basis of a new theoretical view of the immune system. The new model starts with the idea that the driving force for an immune response is not the recognition of a foreign antigen but the recognition of danger. The idea is that incoming viruses, bacteria, worms, and other pathogens create damage in the tissues they inhibit. These tissues relay alarm signals to activate the local sentries (the dendritic cells) to initiate immune responses. The Section is trying to find the tissue-generated alarm signals. It is also studying several implications of the model in such areas as pregnancy, autoimmune disease, and transplantation.

Major Areas of Research

- Immunologic memory
- Danger model of immunity
- Immune tolerance

Selected Recent Publications

Alpan, O., Rudomen, G., Matzinger, P. The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. *J. Immunol.* 166: 4843-52, 2001.

Celli, S., Matzinger, P. Liver transplants induce deletion of liver–specific T cells. *Transplant. Proc.* 33: 102-3, 2001.

Anderson, C.C., Carroll, J.M., Gallucci, S., Ridge, J.P., Cheever, A.W., Matzinger, P. Testing time-, ignorance-, and danger-based models of tolerance. *J. Immunol.* 166: 3663-71, 2001.

Anderson, C.C., Matzinger, P. Immunity or tolerance: opposite outcomes of microchimerism from skin grafts. *Nature Med.* 7: 80-87, 2001.

Gallucci, S., Matzinger, P. Danger signals: SOS to the immune system. *Curr. Opin. Immunol.* 13: 114-9, 2001.

Lantz, O., Grandjean, I., Matzinger, P., DiSanto, J.P. Gamma chain required for naïve CD4+ T cell survival but not for antigen proliferation. *Nature Immunol.* 1: 54-58, 2000.

Gallucci, S., Lolkema, M., Matzinger, P. Natural adjuvants: endogenous activators of dendritic cells. *Nature Med.* 5: 1249-55, 1999.

Laboratory of Clinical Investigation

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Mucosal Immunity Section
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Inflammation Biology Section
Joshua Farber, M.D.

Clinical Studies Unit
Adriana Marques, M.D.

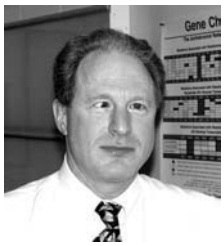
Molecular Microbiology Section
June Kwon-Chung, Ph.D., Head

Immune Cell Interaction Unit
Brian Kelsall, M.D.

Research Activities

The Laboratory of Clinical Investigation (LCI) conducts basic and clinical studies of important human infections and immunologic diseases, with a particular focus on viral, fungal, and bacterial infections, and acquired and congenital immune disorders that may enhance their virulence and chronicity. The program integrates basic cellular and molecular investigation, studies of relevant animal models, and focused analyses of research patients to assemble a comprehensive approach to disease mechanisms and management.

Major current emphases include studies of recurrent herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, and human immunodeficiency virus infection, invasive aspergillosis, cryptococcus, candidiasis, histoplasmosis, Lyme borreliosis, leishmaniasis, and tuberculosis. LCI investigators identify and characterize microbial virulence genes; cellular, cytokine, and chemokine responses to microbial pathogens; and the genetic, cellular, and biochemical bases for immune dysregulation leading to chronic autoimmune and inflammatory diseases and immune deficiency states. A major asset of the LCI is its ability to integrate studies of human subjects to complement and extend observations made in vitro and in cellular and animal model systems. LCI senior staff maintain an extensive portfolio of clinical protocols that serve our clinical, training, and research goals. Immunologic illnesses under active study include inflammatory bowel disease, common variable immunodeficiency, autoimmune lymphoproliferative syndrome, and AIDS.



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Editorial Boards: *Fields Virology*, *The Journal of Virology*, *Virology*, *The Journal of Neurovirology.*

Description of Research Program

Over the past 20 years the research projects in this laboratory have led to the original cloning and mapping of varicella-zoster virus DNA; development and application of molecular epidemiologic tools for studying herpesvirus transmission and reactivation; localization and characterization of the regulation and function of multiple varicella-zoster virus genes; discovery of the transcripts expressed by herpes simplex and varicella-zoster viruses during their latent infection of human neural tissues; and manipulation of herpes simplex virus genes to alter virus reactivation in animal models. The major current bench research emphases remain the molecular and cellular regulation of herpesvirus latency. The laboratory has established ocular and genital herpes models in mice and guinea pigs and used these to explore the viral and host factors that regulate herpes simplex virus (HSV) latency and reactivation. In recent studies we determined that expression of HSV latency transcripts modulate disease reactivation, but the absolute quantity of latent viral DNA in ganglia is the major determinant of reactivation rates. We are using mice deleted for expression of various cytokines to explore immune regulation of latency and reactivation. Most recently, we have created a mouse transgenic for HSV latency-associated transcripts. Ongoing studies are also exploiting animal models to test the efficacy of antiviral drugs, immunoglobulin, and novel DNA-based and genetically engineered vaccines on HSV latency and reactivation.

Major Areas of Research

- Defining the molecular pathogenesis of herpesvirus latency and reactivation
- Development and testing of novel vaccines for herpesviruses

Selected Recent Publications

Quintanilla-Martinez, L., Kumar, S., Fend, F., Reyes, E., Teruya-Feldstein, J., Kingma, D.W., Sorbara, L., Raffeld, M., Straus, S.E., Jaffe, E.S. Fulminant EBV(+) T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome. *Blood* 96: 443-51, 2000.

Lekstrom-Himes, J.A., LeBlanc, R.A., Pesnicak, L., Godleski, M., Straus, S.E. Gamma interferon impedes the establishment of herpes simplex virus type 1 latent infection but has no impact on its maintenance or reactivation in mice. *J. Virol.* 74: 6680-3, 2000.

Marques, A.R., Straus, S.E. Herpes simplex type 2 infections—an update. *Adv. Intern. Med.* 45: 175-208, 2000.

Moriuchi, M., Moriuchi, H., Williams, R., Straus, S.E. Herpes simplex virus infection induces replication of human immunodeficiency virus type 1. *Virology* 278: 534-40, 2000.

Pevenstein, S.R., Williams, R.K., McChesney, D., Mont, E.K., Smialek, J.E., Straus, S.E. Quantitation of latent varicella-zoster virus and herpes simplex virus genomes in human trigeminal ganglia. *J. Virol.* 73: 10514-8, 1999.

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Warren Strober, M.D.

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Dr. Strober is a leading mucosal immunologist who has made a number of important discoveries concerning the function of the mucosal immune system, including the elucidation of IgA B-cell development and the mechanisms of mucosal inflammation. Dr. Strober obtained his medical degree from the University of Rochester and completed an internship and residency at Strong Memorial Hospital in Rochester, NY. He has served as Deputy Scientific Director of the NIAID and as Interim Scientific Director of NIAMS. Dr. Strober is co-editor of major immunologic texts including Current Protocols in Immunology, Clinical Immunologic Principles and Practice, and Mucosal Immunity. He is the recipient of numerous awards including the Distinguished Achievement Award of the American Gastroenterological Association and the PHS Distinguished Achievement Medal. He has served as Chair of the American Board of Allergy and Immunology and is President-Elect of the Society for Mucosal Immunity.

Description of Research Program

Since its inception, Section scientists have maintained an on-going interest in B-cell differentiation particularly relative to the development of the mucosal IgA response. This interest is now accompanied by a major effort in the area of experimental (murine) models of mucosal inflammation, a new area of mucosal immunology that allows study of mucosal immune function both on a basic level and a clinical level. Such models allow broad studies of IL-12 function as well as studies of inflammation that mimic human disease. In the last four years, Section scientists have created new models of mucosal inflammation, analyzed existing models, and have devised new approaches to the treatment of the human counterpart disease, Crohn's disease. One such approach, the treatment of the latter disease with anti-IL-12, is in clinical trial.

Section scientists are clinically oriented and couple their interests in diseases related to mucosal immunity such as the inflammatory bowel diseases, with interests in immunodeficiency diseases, such as common variable immunodeficiency, IgA deficiency, and others. In recent years, the Section has studied the hyper IgM syndrome, elucidating the molecular basis of a new form of this syndrome and conducting a clinical protocol for its treatment. Our broad clinical immunology orientation has led to fruitful interactions with other groups in the LCI, such as the collaborative study of a newly described disease, ALPS.

Major Areas of Research

- Basic studies of mucosal immunity, mucosal inflammation, and inflammatory bowel diseases such as ulcerative colitis and Crohn's disease
- Studies of immunodeficiency such as common variable immunodeficiency and the hyper IgM syndrome; studies of the immunobiology of IL-12
- B-cell differentiation

Selected Recent Publications

Nishikokmori, R., Gurunathan, S., Nishikomori, K., Strober, W. BALB/c mice bearing a transgenic IL-12 receptor beta2 gene exhibit a nonhealing phenotype to Leishmania major infection despite intact IL-12 signaling. *J. Immunol.* 166: 6776-6783, 2001.

Strober, W., Nakamura, K., Kitani, A. The SAMP1/Yit mouse: another step closer to modeling human inflammatory bowel disease. *J. Clin. Invest.* 107: 667-70, 2001.

Jain, A., Ma, C., Liu, S., Brown, M., Cohen, J., and Strober, W. Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. *Nature Immunol.* 2: 223-228, 2001.

Boirivant, M., Fuss, I., Ferroni, L., De Pascale, M., Strober, W. Oral administration of recombinant cholera toxin subunit B inhibits IL-12-mediated murine experimental (trinitrobenzene sulfonic acid) colitis. *J. Immunol.* 166: 3522-3532, 2001.

John Bennett, M.D.

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Education: B.Sc., (chemistry, cum laude), Stanford University; M.D. (Alpha Omega Alpha), Johns Hopkins University School of Medicine. Board certified in Internal Medicine and Infectious Disease. Master, American College of Physicians; former President, Infectious Diseases Society of America; Charter President, Greater Washington Infectious Diseases Society; member, American Society for Clinical Investigation, American Association of Physicians; coeditor of Principles and Practice of Infectious Diseases (five editions); consultant to CDC, ACP-ASIM, USPHA, FDA, DOD.

Description of Research Program

The Clinical Mycology Section has focused on pathogenesis, diagnosis, treatment, prevention, and epidemiology of mycoses, particularly cryptococcosis and candidiasis. Frequent use of fluconazole has led to emergence of fungi with less susceptibility to azoles, most notably *Candida glabrata*. Both primary and secondary resistance occurs in this species. A major mechanism of resistance is azole efflux pumps. We are identifying the efflux pumps and their regulatory control in *C. glabrata*, using isolates from hematopoietic stem cell transplant recipients.

Major Areas of Research

- Azole resistance in *Candida* species
- Infections in hematopoietic stem cell recipients
- Clinical trials of antifungal agents and new diagnostic tests

Selected Recent Publications

Miyazaki, Y., Geber, A., Miyazaki, H., Falconer, D., Parkinson, T., Hitchcock, C., Grimberg, B., Nyswaner, K, and Bennett, J.E. Cloning, sequencing, expression and allelic sequence diversity of ERG3 (C-5 sterol desaturase gene) in *Candida albicans. GENE* 236: 43-51, 1999.

Lamb, D.C., Maspahy, S., Kelly, D.E., Manning, N.J., Geber, A, Bennett, J.E., and Kelly, S.E. Purification, reconstitution and inhibition of cytochrome P-450 sterol ^{22}-desaturase from the pathogenic fungus *Candida glabrata. Antimicrob. Agents Chemother*. 43: 1725-1728, 1999.

Kakeya, H., Miyazaki, Y., Miyazaki, H., Nyswaner, K., Grimberg, B., and Bennett, J.E. Genetic analysis of azole resistance in the Darlington Strain of *Candida albicans. Antimicrob. Agents Chemother.* 44: 2985-2990, 2000.

Graber, C.J., Almeida, K.N.F., Atkinson, J.C., Javaheri, D., Fukuda, C., Gill, V.J., Barrett, A.J., and Bennett, J.E. Dental health and viridans streptococcal bacteremia in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplantation* 27: 537-543, 2001.

Bennett, J.E. Diagnosis and therapy of fungal infections (Ch 200), Histoplasmosis (Ch. 201), Coccidioidomycosis (CH. 202), Blastomycosis (Ch 203), Cryptococcosis (Ch 204), Candidiasis (Ch 205), Aspergillosis (Ch 206), Mucormycosis (Ch. 207) and Miscellaneous mycoses and Algal Infections (Ch. 208), *in* Harrison's Principles of Internal Medicine, 15th Ed, Editors: Braunwald, E., Fauci, A.S., Isselbacher, K.J., Braunwald, E., Kasper, D.L., Hauser, S.L., Longo, D.L., Jameson, J.L. McGraw Hill, New York, 2001.

Bennett, J.E. Antifungal Agents *in* Goodman & Gilman's: The Pharmacologic Basis of Therapeutics, 10th ed. Hardman, J.G., Limbird, L.E., Wonsiecz, M., editors. McGraw Hill, Philadelphia. In press, 2001.

Cortez, K.J., Erdman, D.D., Peret, T.C.T., Gill, V.J., Childs, R., Barrett, A.J., and Bennett, J.E. Outbreak of human parainfluenza virus 3 in a hematopoietic stem cell transplant population. *J. Infect. Dis*. 184: 1093-7, 2001.

Jeffrey I. Cohen, M.D.

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Dr. Cohen received his M.D. from Johns Hopkins, completed an internship and residency at Duke University, worked at NIH studying viral hepatitis, and was an infectious disease fellow at Harvard Medical School working on Epstein-Barr virus. In 1980 he joined the Laboratory of Clinical Investigation. Dr. Cohen is chair of the NIH Biosafety Committee, past chair of the NIH Virology Interest Group, and a member of the American Society for Clinical Investigation.

Description of Research Program

The focus of study is the molecular genetics and pathogenesis of human herpesviruses, principally varicella-zoster virus and Epstein-Barr virus. We have constructed a number of viral mutants, using cosmid systems, and have studied their functions both in vitro and in animal models. The research focuses on viral proteins important for latency, regulation of apoptosis, evasion of the immune system, and interactions with cellular proteins. Viral proteins are studied using in vitro assays, or viruses with mutations in these genes are constructed and studied in cell culture and animal models. Cellular receptors important for herpesvirus binding and fusion to cells are also being identified and characterized. Recombinant herpesviruses are being studied as vectors to deliver novel antigens to the immune system to produce new vaccines. Finally, genetic polymorphisms or mutations are being analyzed to determine their contributions to human herpesvirus infections.

Major Areas of Research

- Pathogenesis of human herpesvirus infection
- Modulation of the immune response by herpesviruses
- Use of herpesviruses as vaccine vectors
- Molecular genetics of herpesviruses

Selected Recent Publications

Cohen, J.I. Epstein-Barr virus infection. *N. Engl. J. Med.* 343: 481-492, 2000.

Soong, W., *et al.* Infection of human T lymphocytes with varicella-zoster virus: an analysis with viral mutants and clinical isolates. *J. Virol.* 74: 1864-1870, 2000.

Cohen, J.I., Lekstrom, K. The Epstein-Barr virus BARF1 protein is dispensable for B cell transformation and inhibits interferon-alpha secretion from mononuclear cells. *J. Virol.* 73: 7627-7632, 1999.

Cohen, J.I., Brunell, P.A., Straus, S.E., Krause, P.R. Recent advances in varicella-zoster virus infection. *Ann. Intern. Med.* 130: 922-932, 1999.

Bertin, J., et al. DED-containing herpesvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. *Proc. Natl. Acad. Sci. USA* 94: 1172-1176, 1997.

Joshua M. Farber, M.D.

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Dr. Farber obtained his M.D. from Johns Hopkins University. Following postdoctoral clinical training in internal medicine and infectious diseases at Johns Hopkins and research training at the NIH and Johns Hopkins, he was on the faculty in the Division of Infectious Diseases at Hopkins before moving to the Laboratory of Clinical Investigation at NIAID in 1993. His research focuses on the role of the chemokine system in inflammation, host defense, and HIV disease.

Description of Research Program

The laboratory investigates the biology of chemokines and their receptors, particularly with regard to their roles in lymphocyte functions relevant to human disease. We have focused on chemokines because they are important in regulating leukocyte trafficking and therefore play a central role in immune responses, and because their receptors are possible targets for therapeutic agonists and/or antagonists. Our approach has been to identify new family members expressed in contexts of interest, and to use molecular tools to investigate the proteins' biological functions. My laboratory discovered chemokines, Mig and the mouse analogue of IP-10 (CRG-2), and receptors, CCR6 and STRL33/CXCR6. We have shown that Mig and other ligands for the Mig receptor, CXCR3, may be particularly important in the trafficking of recently-activated CD4 cells and by implication in the subsequent development of helper/effector function of these cells. We demonstrated that CCR6 is a receptor for MIP-3alpha, a CC chemokine produced by, among other cell types, activated macrophages and endothelial cells, and our data suggest that CCR6 and MIP-3alpha are involved particularly in trafficking of resting memory T cells and activated B cells. We demonstrated, through collaborative efforts, that STRL33/CXCR6 can function as a coreceptor for a variety of strains of HIV-1 and SIV, and that envelope glycoproteins of HIV-1 can signal through chemokine receptors on activated T cells. Our current work focuses on the biology of CXCR3 and CCR6 and their ligands using human cells and gene-targeted mice; the signaling of chemokine receptors on activated lymphocytes; and the role of alternative coreceptors and the chemokine system generally in HIV/AIDS.

Major Areas of Research

- Host defense
- Chemokines and their receptors
- HIV/AIDS

Selected Recent Publications

Yu, C. -R., Peden, K.W.C., Zaitseva, M.B., Golding, H., and Farber, J.M. CCR9A and CCR9B: Two receptors for the chemokine CCL25/TECK/CkBeta-15 that differ in their sensitivities to ligand. *J. Immunol*. 164: 1293-1305, 2000.

Berger, E.A., Murphy, P.M., and Farber, J.M. Chemokine receptors as HIV coreceptors: roles in viral entry, tropism, and disease. *Ann. Rev. Immunol*. 17: 657-700, 1999.

Rabin, R.L., Park, M.K., Liao, F., Swofford, R., Stephany, D., and Farber, J.M. Chemokine receptor responses on T cells are achieved through regulation of both receptor expression and signaling. *J. Immunol*. 162: 3840-3850, 1999.

Liao, F., Rabin, R.L., Smith, C.S., Sharma, G., Nutman, T.B., and Farber, J.M. CCR6 is expressed on diverse memory subsets of T cells and determines responsiveness to mip-3alpha. *J. Immunol*. 162: 186-194, 1999.

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Memberships: American Society for Microbiology; International Society of Human and Animal Mycology; Mycological Society of America; Medical Mycological Society of Americas.

Advisory/Review Boards: Mycology Division of International Union of Microbiological Societies; Molecular Pathogenic Mycology Award Program Advisory Committee, Burroughs Wellcome Fund.
Editorial Board: *Journal of Medical Mycology, Revisita Iberoamericana de Mycologia, FEMS Yeast Research.*
Awards: International Society of Human and Animal Mycology Award; Lucille George's Award; Medical Mycological Society of Americas Award (Rhoda Benham Award); NIH Director's Award; Distinguished Scholar Award, Ewha Woman University; Sung-Ji Award, Korean National Academy of Science; Compatriot Scholar Award, Korea.

Description of Research Program

The program of the Molecular Microbiology Section centers on the molecular dissection of virulence factors (virulence complex) in *Cryptococcus neoformans* and *Aspergillus fumigatus*, which are two of the most common and serious fungal pathogens that infect immunocompromised patients. We have identified several genes that are essential in *C. neoformans* for production of the extracellular polysaccharide capsule and in turn for virulence in experimental animals. These are the first studies that have met the molecular Koch's postulates as applied to fungal pathogenicity. A transcriptional activator, a homolog of *STE12*, was identified as a regulator of the expression of several major virulence factors such as the capsule and laccase genes. A mutation introduced into the homeodomain of the transcriptional activator altered virulence in a mouse model. Structural domains in the *STE12* genes are being investigated for their regulatory effect on several key pathways associated with virulence. Pheromone receptors in *C. neoformans* were observed to sense environmental cues, not only limited to pheromones, which affected virulence in a mouse model. A cluster of six genes involved in the biosynthetic pathway of pentaketide melanin has been cloned from *A. fumigatus*, and their structures, as well as their role in pathogenesis, are under study. All of these studies will allow us to identify potential targets for preventive or therapeutic intervention.

Major Areas of Research

- Biology of pathogenic fungi
- Molecular genetic aspects of *Cryptococcus neoformans* virulence factors
- Pathobiology of *Aspergillus fumigatus*

Selected Recent Publications

Chang, Y.C., Penoyer, L., and Kwon-Chung, K.J. The second STE12 homologue of *Cryptococcus neoformans* is *MAT*α-specific and plays an important role in virulence. *Proc. Natl. Acad. Sci. USA* 98: 3258-3263, 2001

Tsai, H.-F., Fujii, I., Watanabe, A., Wheeler, M.H., Chang, Y.C., Yasuoka, Y., Ebizuka, Y. and Kwon-Chung, K.J. Pentaketide-melanin biosynthesis in *Aspergillus fumigatus* requires chain-length shortening of a heptaketide precursor. *J. Biol. Chem.* 276: 29292-8, 2001.

Karos, M., Chang, Y.C., McClelland, C.M., Clarke, D.L., Fu, J., Wickes, B.L., and Kwon-Chung, K.J. Mapping of the *Cryptococcus neoformans MAT*α locus: presence of mating type-specific pheromone response MAP kinase cascade homologs. *J. Bacteriol.* 182: 6222-6227, 2000.

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Education: Stanford University, B.A. human biology, 1982; Case Western Reserve University School of Medicine, M.D., 1986; Postdoctoral training: The New York Hospital/Cornell Medical Center, 7/86-7/89, Internship, Junior and Senior Resident in internal medicine; University of Virginia Medical Center, 7/89-7/92, Fellowship in infectious diseases; NIH, 9/92-3/96, Fellowship in mucosal immunology.

Description of Research Program

Stimulation of T-helper (Th) cells and naive B cells in the Peyer's patches of the small intestine with orally administered antigens leads to the induction and dissemination of IgA-producing B and Th cells to mucosal effector tissues, such as the lamina propria of the GI and upper respiratory tracts, and to secretory glands for subsequent antigen-specific secretory IgA antibody responses. At the same time, however, systemic T- and B- cell immune responses to the same antigen may be suppressed– a phenomenon known as oral tolerance. This ability of oral antigens to both stimulate mucosal and suppress systemic immune responses likely involves antigen processing and presentation in the Peyer's patches (PP)/lymphoid follicles of the intestine, which have associated epithelial cells (M-cells), specialized for the sampling and transport of luminal antigens.

The main objectives of our work to date have been the following: 1) to understand how protein antigens are processed and presented in the murine Peyer's patch and how this is related to oral tolerance and IgA B cell development; 2) to elucidate the role of cytokines, particularly IL-12, in the generation of oral tolerance and immunization; 3) to characterize the regulation of IL-12 production by antigen presenting cells that may be important in mucosal immune responses; 4) based on knowledge from these studies to develop novel immunization strategies for the stimulation of protective mucosal immune responses and induction of oral tolerance, and to develop novel methods of immunomodulation for Th1-mediated intestinal inflammation.

Major Areas of Research

- Mucosal immunology
- Dendritic cells
- Regulation of IL-12 production

Selected Recent Publications

Marth, T.M. and Kelsall, B.L. Regulation of IL-12 by complement receptor 3 signaling. *J. Exp. Med.* 185: 1987-1995, 1997.

Iwasaki, A., and Kelsall, B.L. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J. Exp. Med.* 190:229-39, 1999.

Braun, M. and Kelsall, B.L. Selective suppression of IL-12 production by chemoattractants. *J. Immunol*. 164: 3009-3017, 2000.

Iwasaki, A., and Kelsall, B.L. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines MIP-3α, MIP-3β and SLC. *J. Exp. Med.* 191:1381-1393, 2000.

He, J., Gurunathan, S., Ash-Shaheed, B., and Kelsall, B.L. Major role for Gi-protein signaling in the regulation of Th1 immune responses. *J. Exp. Med.* 191: 1605-1610, 2000.

Braun, M.C., Wang, J.M., Rabin, R., Lahey, E., and Kelsall, B.L. Activation of chemotactic receptor FPR by the HIV-derived peptide T-20 suppresses IL-12 p70 production by human monocytes. *Blood* 97: 3531-6, 2001.

Iwasaki, A., Ash-Shaheed, B., and Kelsall, B.L. Unique features of Peyer's patch lymphoid, myeloid, and double negative dendritic cells. *J. Immunol.* 166: 4884-4890, 2001.

Adriana Marques, M.D.

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Dr. Marques received her medical degree from the Federal University of Rio Grande do Sul, Brazil. She subsequently completed internal medicine residency at the Hospital de Clinicas de Porto Alegre, followed by subspecialty training in pulmonology at the Federal University of Rio Grande do Sul. She joined NIAID's intramural research program in 1993, when she was awarded a postdoctoral fellowship in infectious diseases. In 1995, she was selected to direct a new clinical research initiative for LCI, and in 1997 was promoted to Head of the Clinical Studies Unit.

Description of Research Program

The Clinical Studies Unit (CSU) was created in 1997 to coalesce into one administrative structure the LCI staff who conduct clinical research. The Unit has as its main objective the development and conduct of clinical research in areas of interest to the Laboratory of Clinical Investigation.

Major Areas of Research

- Lyme disease
- Herpes simplex virus infections
- Herpes zoster virus infections

Selected Recent Publications

Hemmer, B., Gran, B., Zhao, Y., Marques, A., Pascal, J., Tzou, A., Kondo, T., Cortese, I., Bielekova, B., Straus, S., McFarland, H., Houghten, R., Simon, R., Pinilla, C., Martin, R. Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease. *Nature Med.* 5: 1375-1382, 1999.

Liang, F.T., Steere, A.C., Marques, A., Miller, J., Johnson, B., Philip, M.T. Sensitive and specific serodiagnosis of Lyme disease by ELISA with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi* VlsE. *J. Clin. Microbiol.* 37: 3990-3996, 1999.

Marques, A., Straus, S.E. Herpes simplex type 2 infections – an update. *Advances of Internal Medicine* 45: 175-208, 2000.

Marques, A. Lyme Disease. In: MKSAP 12. American College of Physicians. 2001.

Laboratory of Host Defenses

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Molecular Signaling Section
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Monocyte Trafficking Unit
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Eosinophil Biology Unit
Helene F. Rosenberg, M.D., Ph.D., Head

Clinical Pathophysiology Section
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Research Activities

The Laboratory of Host Defenses studies mechanisms of host defense against bacterial and fungal infections, with particular emphasis on the biochemistry, structure, and function of phagocytic cells, including peripheral blood polymorphonuclear leukocytes (neutrophils), eosinophils, monocytes, and fixed-tissue macrophages. The program integrates laboratory studies with the development of diagnostic and therapeutic approaches to patients in whom inherited defects in host defense affect phagocytic cells. Clinical and clinically related studies include the development of diagnostic tools and treatments such as gene therapy and bone marrow transplantation for chronic granulomatous diseases (CGD) of childhood; delineation of the structure and function of the NADPH oxidase enzyme that is defective in CGD; delineation of inherited defects in host defense leading to infections with atypical mycobacteria; treatment of atypical mycobacteria infections and multidrug resistant tuberculosis with novel cytokines such as interferon gamma and interleukin-12; delineation of the structure and biology of chemokine receptors involved in host defense against HIV; delineation of the role of chemokine receptors in host defense and the regulation of eosinophil development; studies of the role of eosinophil ribonucleases in host defense against single-stranded RNA viruses; studies of the biology of human stem cells as targets for gene therapy of immune system disorders; and delineation of the role of cytokines such as interferon gamma, interleukin-8, interleukin-4, and tumor necrosis factor in the function of phagocytic cells and the process of inflammation.

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Dr. Gallin earned his M.D. at Cornell University Medical College and then established a career in biomedical research at NIH as an active clinician, researcher, and administrator. He served as Scientific Director, NIAID, from 1985 to 1994 and provided oversight of all intramural activities for NIAID, including a doubling of the research budget in response to the AIDS epidemic. Since 1991, Dr. Gallin has been Chief of the Laboratory of Host Defenses with a primary research focus on the role of phagocytes. His Laboratory studies the genetic basis for several forms of chronic granulomatous disease (CGD), a rare hereditary immune disorder, and the use of interferon-gamma to reduce life-threatening infections in CGD. In 1994, Dr. Gallin was appointed Director of the NIH Warren Grant Magnuson Clinical Center and has led the revitalization of clinical research at NIH. He has authored more than 275 research articles, has received numerous distinguished awards, and has served on numerous editorial boards. Dr. Gallin is a member of the Institute of Medicine of the National Academy of Sciences.

Description of Research Program

Long-term studies are performed on natural history and disease pathogenesis on patients with abnormal phagocyte function. These patients include those with CGD of childhood, hyperimmunoglobulin E-recurrent infection (Job's) syndrome, and leukocyte adhesion deficiency as well as those with recurrent infections that do not fall into a specifically defined disease category. Specific studies include function of phagocytic cells and production of cytokines such as IL-8.

Specific emphasis is placed on the study of the etiology, pathogenesis, and therapy of granulomatous disorders caused by an inherited defect in the ability of phagocytes (neutrophils and monocytes) to produce superoxide. This defect leads to recurrent life-threatening bacterial and fungal infections as well as tissue granuloma formation. The phagocyte-stimulating cytokine interferon-gamma (IFNγ) has been shown to reduce the frequency and severity of infections in CGD. The Laboratory has created a mouse model of the most common autosomal recessive form of CGD. This knockout mouse faithfully mimics the human disease and is being used to study the multiple roles of superoxide in the inflammatory process and other processes. In addition, the effects and mechanisms of cytokines in this setting are studied.

Mycobacterium tuberculosis infects more than one-third of humankind and is the leading cause of infectious disease mortality worldwide. The research goals are to understand and enhance the host factors responsible for resistance to *Mycobacteria.* These goals are being pursued through study of the macrophage responses of patients with susceptibility to low-level mycobacterial pathogens such as *Mycobacterium avium* complex. In addition, Laboratory scientists are treating patients with mycobacterial infections with IFN-γ to enhance mycobacterial killing.

Major Areas of Research

- Abnormal phagocyte function
- Etiology, pathogenesis, and therapy of granulomatous disorders
- Resistance to *Mycobacteria* infection

Selected Recent Publications

Lekstrom-Himes, J.A., Gallin, J.I., Immunodeficiency diseases caused by defects in phagocytes. *N. Engl. J. Med.* 343: 1703-1714, 2000.

Lekstrom-Himes, J.A., Dorman, S.E., Kopar, P., Holland, S.M., Gallin, J.I. Neutrophil-specific granule deficiency results from a novel mutation with loss of function of the transcription factor CCAAT/enhancer protein epsilon. *J. Exp. Med.* 189: 1847-1852, 1999.

Harry Malech, M.D.

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Dr. Malech received his M.D. from Yale University School of Medicine in 1972. Following an internal medicine residency at the Hospital of the University of Pennsylvania, he was a Research Fellow in the Laboratory of Cell Biology, NCI. He then obtained postdoctoral fellowship training in infectious diseases at Yale University School of Medicine. In 1978, Yale University School of Medicine appointed him Assistant Professor and in 1982 promoted him to Associate Professor of Medicine. In 1986, Dr. Malech returned to NIH as the Head of the Bacterial Diseases Section, LCI, NIAID. In 1992, NIAID established the Laboratory of Host Defenses with Dr. Malech as Deputy Chief and Head of LHD's Genetic Immunotherapy Section. Dr. Malech serves on a number of editorial boards and NIH committees.

Description of Research Program

Dr. Malech has focused his laboratory and clinical research work on inherited defects in immunity, with particular emphasis on chronic granulomatous disease (CGD), a defect in the microbicidal oxidative function of phagocytic cells. His laboratory identified and cloned two of the genes responsible for the four genetic types of CGD. His clinical studies have encompassed the molecular genetics, the diagnosis, and the treatment of CGD and other immune disorders. In 1993 Dr. Malech established a program focused on the development of gene therapy for CGD, targeting hematopoietic stem cells, and this led to clinical trials of gene therapy for both autosomal recessive and X-linked CGD. This program encompasses the biology of human hematopoietic stem cells, which includes studies of the mobilization, purification, and culture of peripheral blood stem cells. More recently, Dr. Mitchell Horwitz, a transplant specialist in Dr. Malech's group has expanded this program to include a clinical trial of a non-ablative conditioning approach to allogeneic stem cell transplantation for CGD. Also, in collaboration with Dr. Jennifer Puck of NHRGI, Dr. Malech has expanded his gene therapy program to include the development and conduct of a clinical trial for X-linked severe combined immune deficiency (X-SCID), a severe defect in T and B lymphocyte and NK cell function caused by a defect in the gene encoding the common gamma chain shared by the receptors for interleukins 2, 4, 7, 9, and 15.

Major Areas of Research

- Inherited defects in immunity
- Chronic granulomatous disease

Selected Recent Publications

Horwitz, M.E., Barrett, A.J., Brown, M.R., Carter, C.S., Childs, R., Gallin, J.I., Holland, S.M., Linton, G.F., Miller, J.A., Leitman, S.F., Read, E.J., Malech, H.L. Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft. *N. Engl. J. Med.* 344: 881-888, 2001.

Aviles Mendoza, G.J., Seidel, N.E., Otsu, M., Anderson, S.M., Simon-Stoos, K., Herrera, A., Hoogstraten-Miller, S., Malech, H.L., Candotti, F., Puck, J.M., Bodine, D.M. Comparison of five retrovirus vectors containing the human IL-2 receptor gamma chain gene for their ability to restore T and B lymphocytes in the x-linked severe combined immunodeficiency mouse model. *Mol. Ther.* 3: 565-573, 2001.

Rosenzweig, M., MacVittie, T.J., Harper, D., Hempel, D., Glickman, R.L., Johnson, R.P., Farese, A.M., Whiting-Theobald, N., Linton, G.F., Yamasaki, G., Jordan, C.T., and Malech, H.L. Efficient and durable gene marking of hematopoietic progenitor cells in nonhuman primates after nonablative conditioning. *Blood* 94: 2271-2286, 1999.

Malech, H.L., Maples, P.B., Whiting-Theobald, N., Linton, G.F., Sekhsaria, S., Vowells, S.J., Li, F., Miller, J.A., DeCarlo, E, Holland, S.M., Leitman, S.F., Carter, C.S., Butz, R.E., Read, E.J., Fleisher, T.A., Schneiderman, R.D., Van Epps, D.E., Spratt, S.K., Maack, C.A., Rokovich, J.A., Cohen, L.K., and Gallin, J.I. Prolonged production of NADPH oxidase-corrected granulocytes following gene therapy of chronic granulomatous disease. *Proc. Natl. Acad. Sci. USA* 94: 12133-12138, 1997.

Steven M. Holland, M.D.

Head, Immunopathogenesis Unit, LHD

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Dr. Holland received his M.D. degree and training in internal medicine and infectious diseases from the Johns Hopkins University School of Medicine in 1983. He served as an Assistant Chief of Service in the Department of Medicine at that institution. He joined NIH in 1989 as a National Research Council Fellow in the LMM. In 1991, Dr. Holland joined the LHD, where his work centers on immune defects affecting phagocytes with emphasis on cytokines in the etiology, pathogenesis, and therapy of infections. His laboratory studies mycobacterial susceptibility in patients with severe nontuberculous and tuberculous mycobacterial infections, especially those defects associated with interferon gamma and interleukin-12 synthesis and response.

Description of Research Program

Studies focus on the etiology, pathogenesis, and therapy of granulomatous disorders. Mycobacteria infect more than one-third of the world's population and are the leading cause of infectious disease mortality worldwide. The Unit is interested in understanding and enhancing the host factors responsible for resistance to mycobacteria and is pursuing these goals through study of the macrophage responses of patients with susceptibility to mycobacteria of low intrinsic virulence such as *Mycobacterium avium* complex (MAC). In addition, staff members treat patients with mycobacterial infections with interferon gamma or interleukin-12 to enhance mycobacterial killing.

Chronic granulomatous disease (CGD) is caused by an inherited defect in the ability of phagocytes (neutrophils and monocytes) to produce superoxide. This defect leads to recurrent life-threatening bacterial and fungal infections as well as tissue granuloma formation. The phagocyte-stimulating cytokine interferon gamma has been shown to reduce the frequency and severity of infections in CGD. The Unit created a mouse model of the most common autosomal recessive form of CGD by homologous recombination. This knockout mouse faithfully mimics the human disease and is being used to study the multiple roles of superoxide in inflammatory and other processes. In addition, the effects and mechanisms of cytokines in this setting are studied.

Major Areas of Research

- Immune defects of phagocytes and role of cytokines in pathogenesis and therapy of infections
- Susceptibility to mycobacterial infections

Selected Recent Publications

Dupuis, S., Dargemont, C., Fieschi, C., Thomassin, N., Rosenzweig, S., Harris, J., Holland, S.M., Schreiber, R.D., Casanova, J.L. Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. *Science* 293: 300-3, 2001.

Lavigne, M.C., Malech, H.L., Holland, S.M., Leto, T.L. Genetic demonstration of p47phox-dependent superoxide anion production in murine vascular smooth muscle cells. *Circulation* 104: 79-84, 2001.

Sereti, I., Holland, S.M. Disseminated nocardiosis in a patient with x-linked chronic granulomatous disease and human immunodeficiency virus infection. *Clin. Infect. Dis.* 33: 235-9, 2001.

Fieschi, C., Dupuis, S., Picard, C., Smith, C.I., Holland, S.M., Casanova, J.L. High levels of interferon gamma in the plasma of children with complete interferon gamma receptor deficiency. *Pediatrics* 107: E48, 2001.

Holland, S.M. Immunotherapy of mycobacterial infections. *Semin. Respir. Infect.* 16: 47-59, 2001.

Lavigne, M.C., Malech, H.L., Holland, S.M., Leto, T.L. Genetic requirement of p47phox for superoxide production by murine microglia. *FASEB J.* 15: 285-7, 2001.

Thomas L. Leto, Ph.D.

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Dr. Leto received his Ph.D. in biochemistry from the University of Virginia for studies on mechanisms of cell membrane assembly. He followed this work with postdoctoral studies at Yale University on membrane-cytoskeleton interactions. Dr. Leto joined the NIAID in 1988 and became a Senior Investigator in the Laboratory of Host Defenses in 1996. His research focuses on the production of reactive oxygen species by phagocytic and non-phagocytic cells, and its role in host defense, inflammation, and cell signaling.

Description of Research Program

This group studies structure-function relationships in a family of NADPH oxidases responsible for generation of reactive oxygen species. Early work has focused on the enzyme in phagocytic blood cells (phox, or phagocyte oxidase) and has included studies on structural mutations detected in chronic granulomatous disease; regulatory proteins that affect oxidase function (Ras-related proteins, Src homology domains, phospholipases, and protein kinases); and signal transduction processes involved in neutrophil activation. Studies in phox-deficient mice also demonstrated a role for this enzyme in release of reactive oxygen species by vascular smooth muscle cells and microglia. Recent accomplishments include the demonstration that assembly of activated NADPH oxidase involves interactions of Src homology 3 domains with proline-rich targets in other oxidase components. Phospholipases A2 and D were also implicated in phagocyte activation in transfected cell models. These observations may serve as a basis for drugs designed to limit or augment oxidant production at sites of inflammation or infection.

Other recent directions include characterization of sequence-related phox homologues that serve as sources of reactive oxidants in a variety of other tissues, most notably the kidney and colon. Genetic approaches are being used to explore proposed functions of these enzymes (oxygen sensing, host defense, proliferation, differentiation, and cellular senescence).

Major Areas of Research

- NADPH oxidases
- Signal transduction in phagocytic cells
- Role of reactive oxygen in health and disease (host defense, inflammation, signal transduction)

Selected Recent Publications

Lavigne, M.C., Malech, H.L., Holland, S.M., and Leto, T.L. Genetic requirement of p47phox for superoxide production by murine microglia. *FASEB J.* 15:285-287, 2001.

Dana, R.R., Eigsti, C., Holmes, K.L., and Leto, T.L. A regulatory role for ADP-ribosylation factor 6 (ARF6) in activation of the phagocyte NADPH oxidase. *J. Biol. Chem.* 275: 32566-32571, 2000.

Geiszt, M., Kopp, J.B., Varnai, P., and Leto, T.L. Identification of renox, an NAD(P)H oxidase in kidney. *Proc. Natl. Acad. Sci. USA* 97: 8010-8014, 2000.

Philip M. Murphy, M.D.

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Dr. Murphy obtained an A.B. from Princeton in 1975. He received an M.D. from Cornell in 1981 and trained in internal medicine at New York University through 1985 as intern, resident and chief resident. Following subspecialty fellowship training in infectious diseases at NIH, he joined the Laboratory of Host Defenses, NIAID, where he earned tenure in 1992. In 1998 he was promoted to the Senior Biomedical Research Service, NIH, and named Head of the Molecular Signaling Section, LHD. Dr. Murphy's research interests include the molecular mechanisms of leukocyte chemotaxis, inflammation and AIDS.

Description of Research Program

The Molecular Signaling Section, LHD studies mechanisms of host defense and inflammation, focusing on the molecular basis of leukocyte activation by chemoattractants, including the chemokines. Genes have been isolated for a large number of chemoattractant receptors that are differentially expressed in blood leukocytes. One of the first ones found was the interleukin-8 receptor, which is most highly expressed in neutrophils and appears to be important in acute inflammation. More recently, four monocyte-selective chemokine receptors and an eosinophil-selective chemokine receptor have been cloned that are believed to play roles in chronic and allergic inflammation. We hope to establish the precise importance of each of these receptors *in vivo* by making targeted disruptions of the corresponding genes in mice and by studying the distribution of naturally occurring mutant receptors in specific human disease contexts. To date we have used these approaches to identify potential roles for chemokine receptors in HIV/AIDS, atherosclerotic heart disease and Alzheimer's disease. The results have the potential for clinical application in these diseases through discovery and development of receptor antagonists.

Although chemoattractant receptors probably evolved to support antimicrobial host defense, we and others have discovered that certain receptors have been exploited by microbial pathogens as pro-microbial factors. Two modes of exploitation have been identified. In the first, taxonomically diverse obligate intracellular pathogens use cellular chemoattractant receptors encoded by the host organism as essential factors for target cell entry. Examples include *Plasmodium vivax* and HIV-1, which exploit specific chemokine receptors. A distinct second mode of exploitation involves virally encoded chemoattractant receptors. Examples are chemokine receptors encoded by three herpesviruses: human cytomegalovirus, Kaposi's sarcoma-associated herpesvirus and *Herpesvirus saimiri*. The corresponding viral genes were apparently copied from host genes in a process analogous to viral oncogenes. Future identification of the biological function of the viral receptors could lead to novel antiviral therapies and new insights regarding the precise biological functions of the mammalian homologues.

Major Areas of Research

- Host defense and inflammation
- Chemoattractant receptors

Selected Recent Publications

Tiffany, H.L., Lavigne, M.C., Cui, Y-H, Wang, J-M, Leto, T.L., Gao, J-L and Murphy, P.M. Amyloid-beta induces chemotaxis and oxidant stress by acting at formylpeptide receptor 2 (FPR2), a G protein-coupled receptor expressed in phagocytes and brain. *J. Biol. Chem.* 276:23645-52, 2001.

Schwarz, M. and Murphy, P.M. Kaposi's sarcoma-associated herpesvirus (KSHV) G protein-coupled receptor constitutively activates NFκB and induces pro-inflammatory cytokine and chemokine production via a C-terminal signaling determinant. *J. Immunol.* 167:505-513, 2001.

Helene F. Rosenberg, M.D., Ph.D.

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Dr. Rosenberg obtained her M.D. and Ph.D. degrees from the joint program at The Rockefeller University and Cornell University Medical College. Following postdoctoral research at Harvard University, she joined the Laboratory of Host Defenses in 1991, and became Head of the Eosinophil Biology Unit in 1998.

Description of Research Program

The primary focus of our laboratory program is the eosinophil, an enigmatic leukocyte whose role in host defense remains a subject of concern and controversy. Among the toxic proteins secreted by activated eosinophils are two ribonucleases known as eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN). A number of studies from our laboratory have led us to consider the role of these ribonucleases, and by extension, the role of their primary host cells, the eosinophils, in host defense against a previously unrecognized group of target pathogens, specifically respiratory viruses of the family *Paramyxoviridae*. We have developed a novel mouse model of respiratory viral infection using the natural paramyxovirus pathogen of rodents, pneumonia virus of mice. With this model, we are investigating the role of eosinophils in host defense against these clinically important respiratory viruses and directing our studies toward the elucidation of novel immunomodulatory therapies. Currently, members of the Eosinophil Biology Unit include: Kimberly D. Dyer, Ph.D., Jianzhi George Zhang, Ph.D., Joseph B. Domachowske, M.D. (SUNY Syracuse), Joanne Moreau, B.S., and Nora L. Vasquez, B. S.

Major Areas of Research

- Eosinophils, eosinophil ribonuclease and innate immunity against respiratory viruses
- Molecular biology and evolution of the Ribonuclease A gene superfamily
- Eosinophil trafficking and function in respiratory viral infection

Selected Recent Publications

Zhang, J., Dyer, K.D., and Rosenberg, H.F. Evolution of the rodent eosinophil-associated ribonuclease gene family by rapid gene sorting and positive selection. *Proc. Natl. Acad. Sci.* USA 97:4701-4706, 2000.

Domachowske, J.B., Bonville, C.A., Gao, J.L., Murphy, P.M., Easton, A.J., and Rosenberg, H.F. The chemokine MIP-1alpha and its receptor CCR1 control pulmonary inflammation and anti-viral host defense in paramyxovirus infection. *J. Immunol.* 165: 2677-2682, 2000.

Rosenberg, H.F., Domachowske, J.B. Eosinophils, ribonucleases and host defense: solving the puzzle. *Immunologic Research* 20: 261-274, 1999.

Rosenberg, H.F. *Eosinophils*, in Inflammation: Basic Principles and Clinical Correlates, 3rd Edition (Gallin, J.I., *et al.*, eds.), Raven Press Ltd. New York, NY, pp. 65-76, 1999.

Domachowske, J.B., Dyer, K.D., Bonville, C.A., and Rosenberg, H.F. Recombinant human eosinophil-derived neurotoxin / RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. *J. Infect. Dis.* 177:1458-1454, 1998.

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Investigator, LHD

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Dr. Jackson received her M.D. from the State University of New York at Buffalo School of Medicine in 1988. She completed a pediatric residency at Mount Sinai Hospital in New York, and subspecialty training in allergy and immunology at the National Institute of Allergy and Infectious Diseases, NIH. Dr. Jackson joined the Laboratory of Host Defenses, NIAID, in 1992 for the research component of her allergy and immunology fellowship, during which she developed the autosomal recessive $p47^{phox}$ knock-out mouse model of chronic granulomatous disease (CGD). Since 1996 her research has focused on mechanisms for induction of host resistance to infection and inflammation.

Description of Research Program

The research in this laboratory focuses on characterizing the functional relationships between innate immune factors and antigen presenting cells (dendritic cells, monocytes/ macrophages), and defining the *in vivo* trafficking patterns, at rest and during disease states, of immature and mature dendritic cells.

Recent accomplishments include developing a novel *ex vivo* culture system for proliferating and differentiating large numbers of dendritic cells from defined murine bone marrow progenitors (Sca-1^+ cells) using a combination of myeloid (GM-CSF, M CSF) and stromal (SCF, IL-3, Flt-3L) growth factors. The *in vitro* model delineates a complete differentiation pathway from the Sca-1^+ progenitor cell to a mature dendritic cell; including the generation of dendritic cells at three distinct stages of differentiation: precursor, immature and functionally mature. The model also defines immunomodulatory cytokines and innate immune factors that mediate the process.

Future interests include delineating the role of dendritic cells in the pathogenesis of host resistance to infection and inflammatory diseases states, and developing dendritic cell-based immunotherapies.

Major Areas of Research

- Dendritic cell biology and function
- Myeloid cell trafficking
- CGD

Selected Recent Publications

Jackson, S.H., Miller, G.F., Mardiney III, M., Domachowske, J.D., Gallin, J.I. and Holland, S.M. Interferon-γ is effective in the mouse model of chronic granulomatous disease. *Journal of Interferon and Cytokine Research* 21: 567-73, 2001.

Hentunen, T.A., Jackson, S.H., Chung, H., Reddy, S.V., Lorenzo, J., Choi, S.J. and Roodman, G.D. Characterization of immortalized osteoclast precursors developed from mice transgenic for both BCL-XL and simian v40 large T antigen. *Endocrinology* 140: 2954-61, 1999.

Laboratory of Human Bacterial Pathogenesis

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Frank R. DeLeo, Ph.D.

B. Joseph Hinnebusch, Ph.D.

Michael Otto, Ph.D.

Research Activities

The Laboratory of Human Bacterial Pathogenesis (LHBP) studies the molecular basis of human bacterial pathogenesis in its broadest sense. Research projects currently focus on the molecular basis of pathogen-arthropod vector interaction using pathogenic *Borrelia* species-tick and *Yersinia pestis*-flea as model systems; the role of high-frequency genetic and antigenic variation in the pathogenesis of *Neisseria gonorrhoeae* infections; the genetic basis of antimicrobial agent resistance, host susceptibility, and disease specificity in *Mycobacterium tuberculosis;* and the molecular basis of epidemic waves and human-pathogen interactions in group A *Streptococcus.* High-throughput automated DNA sequencing strategies, *in vitro* tissue culture, molecular genetic approaches, and animal models are used to assist these investigations.

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Dr. Musser received his M.D. and Ph.D. from the University of Rochester School of Medicine. Following postdoctoral research at the Institute of Molecular Evolutionary Genetics, Pennsylvania State University, and residency training in laboratory medicine at the Hospital of the University of Pennsylvania, he joined the Department of Pathology, Baylor College of Medicine, Houston, Texas. He advanced through the academic ranks from 1991 to 1998, when he was promoted to Professor. Dr. Musser joined NIAID in 1999 as Chief, Laboratory of Human Bacterial Pathogenesis. His research focuses on the molecular basis of host-pathogen interactions in group A *Streptococcus* and *Mycobacterium tuberculosis*. He serves on several editorial boards and has received many national and international honors and awards.

Description of Research Program

The long-term goals of research conducted in the laboratory are to understand the molecular basis of diseases caused by the human pathogenic bacterium Group A *Streptococcus* (GAS) and elucidate the molecular basis underlying variation in the character of disease caused by *Mycobacterium tuberculosis.* Genome-scale and high-throughput strategies are used to investigate (1) the role of extracellular GAS proteins in host-pathogen interactions, (2) potential immunoprophylaxis applications of these proteins, and (3) the molecular pathogenesis of rheumatic fever and rheumatic heart disease. In *M. tuberculosis*, the laboratory is addressing (1) the molecular genetic basis of antimicrobial agent resistance, (2) the genetics of disease specificity recently identified for certain *M. tuberculosis* clones, and (3) the human genetics of susceptibility to this pathogen. To address these issues, a highly integrated approach is used that encompasses bacterial molecular genetics, genome sequencing, molecular population genetic analysis, and human genetic epidemiology.

Major Areas of Research

- Group A *Streptococcus*
- *Mycobacterium tuberculosis*

Selected Recent Publications

Reid, S.D., Green, N.M., Buss, J.K., Lei, B., Musser, J.M. Multilocus analysis of extracellular putative virulence proteins made by group A *Streptococcus*: population genetics, human serologic response, and gene transcription. *Proc. Natl. Acad. Sci. USA* 98: 7552-7, 2001.

Fitzgerald, J.R., Sturdevant, D.E., Mackie, S.M., Gill, S.R., Musser, J.M. Evolutionary genomics of *Staphylococcus aureus*: Insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *Proc. Natl. Acad. Sci. USA* 98: 8821-6, 2001.

Reid, S.D., Hoe, N.P., Smoot, L.M., Musser, J.M. Group A *Streptococcus*: allelic variation, population genetics, and host-pathogen interactions. *J. Clin. Invest.* 107: 393-9, 2001.

Hoe, N.P., Vuopio-Varkila, J., Vaara, M., Grigsby, D., De Lorenzo, D., Fu, Y.X., Dou, S.J., Pan, X., Nakashima, K., Musser, J.M. Distribution of streptococcal inhibitor of complement variants in pharyngitis and invasive isolates in an epidemic of serotype M1 group A *Streptococcus* infection. *J. Infect. Dis.* 183:633-9, 2001.

Smoot, L.M., J.C. Smoot, M.R. Graham, G.A. Somerville, D.E. Sturdevant, C.A. Lux Migliaccio, G.L. Sylva, and J. M. Musser. Global differential gene expression in response to growth temperature alteration in group A *Streptococcus*. *Proc. Natl. Acad. Sci. USA* 98: 10416-21, 2001.

Robert J. Belland, Ph.D.

Investigator, LHBP

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B.Sc., Biochemistry, University of Victoria, Victoria, B.C., Canada; Ph.D., Biochemistry, University of Victoria, Victoria, B.C., Canada; Visiting Associate, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories (1990-1998); Investigator, Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories (1998-present).

Description of Research Program

Understanding the pathogenesis of chlamydial disease has been difficult for a variety of reasons including the obligate intracellular nature of the organisms and the lack of established genetic systems. I have chosen to study the human pathogens *Chlamydia trachomatis* and *Chlamydia pneumoniae* using a genomic approach in order to understand, at the molecular level, the patterns of bacterial gene expression during the course of human disease. Complete cDNA microarrays have been made for each of the organisms and are being used to define the bacterial developmental cycle in transformed cell lines of epithelial origin (HeLa 229). These studies are being done in parallel with an analysis of the host-cell gene expression changes during the infectious cycle to determine how the pathogen directs the remodeling of the host-cell environment. These studies have defined a number of new 'early' and 'late' cycle genes that may be important targets of immunologic or therapeutic agents. We have begun to characterize the means by which the host cell controls chlamydial growth (i.e. production of IFNγ) by determining gene expression patterns under these conditions and comparing them to the baseline studies of normal bacterial growth. The goal of these studies is to gain an understanding of how the organism enters and maintains a 'persistent' growth state, a condition linked to more severe forms of human disease (i.e. the association of *C. trachomatis* with cervical carcinoma and the association of *C. pneumoniae* with atherosclerotic disease).

Comparative genomic analysis of the 'plasticity zone' of *C. trachomatis* has pointed to a new, potential virulence factor for this organism. The gene in question encodes a very large protein with significant homology to a number of bacterial toxins, primarily the A and B toxins of *Clostridium difficile*. We have shown that expression of the protein results in the phenomenon known in the literature as 'immediate cytotoxicity' in which the host-cell cytoskeleton is disrupted due to microfilament depolymerization. Laboratory-adapted bacterial strains appear to undergo genetic deletion events in the toxin gene during initial isolation resulting in a number of partial open reading frames which give rise to attenuated toxin products. While the role of this toxin in pathogenesis of chlamydial disease may be complicated, we feel it is an important new area of chlamydial pathogenesis research.

Major Areas of Research

- Chlamydial genomics
- Chlamydia pathogenesis
- Persistent bacterial infections

Selected Recent Publications

This is a new project, previous publications include:

Bos, M. P., Kuroki, M., Krop-Watorek, A., Hogan, D., and Belland, R. J. CD66 receptor specificity exhibited by neisserial Opa variants is controlled by protein determinants in CD66 N-domains. *Proc. Natl. Acad. Sci. USA* 95: 9584-9589, 1998.

Bos, M. P., Hogan, D., and Belland, R. J. Homolog scanning mutagenesis reveals CD66 receptor residues required for neisserial Opa protein binding. *J. Exp. Med.* 190: 331-340, 1999.

Frank R. DeLeo, Ph.D.

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Dr. DeLeo received his Ph.D. in microbiology from Montana State University in 1996 and has spent the last 8 years studying human polymorphonuclear leukocytes (PMNs) and how they mediate the innate immune response. Dr. DeLeo joined the staff at Rocky Mountain Laboratories in the fall of 2000 as an Investigator in the Laboratory of Human Bacterial Pathogenesis.

Description of Research Program

Dr. DeLeo's laboratory is interested in understanding the molecular basis of the interaction of pathogenic bacteria with human phagocytic leukocytes. His expertise encompasses the phagocyte NADPH-dependent oxidase, phagocytosis and neutrophil function in general. His laboratory is a recent addition to the Laboratory of Human Bacterial Pathogenesis and is currently applying genomics and proteomics methodologies to identify specific genes and/or proteins in bacteria that mediate immune subversion by their ability to escape destruction by human phagocytes. The laboratory also employs standard cell biological and biochemical approaches to elucidate the responses of human PMNs to bacterial pathogens. Projected studies include genome-scale differential gene expression studies in microorganisms such as Group A *Streptococcus* and *Staphylococcus aureus* during phagocytosis.

Major Areas of Research

- Biology and function of human PMNs
- Neutrophil-pathogen interactions, including phagocytosis, and oxidative and non-oxidative bactericidal activity of PMNs toward specific microbes
- Identification of genes/proteins up-regulated during phagocytosis of bacterial pathogens
- Identification of proteins critical for PMN responses to microbes

Selected Recent Publications

Burritt, J.B., DeLeo, F.R., McDonald, C.L., Prigge, J.R., Dinauer, M.C., Nakamura, M., Nauseef, W.M., and Jesaitis, A.J. Phage display epitope mapping of human neutrophil flavocytochrome b_{558}. Identification of two juxtaposed extracellular domains. *J. Biol. Chem*. 276: 2053-61, 2001.

DeLeo. F.R., Burritt, J.B., Yu, L., Jesaitis, A.J., Dinauer, M.C., and Nauseef, W.M. Processing and maturation of flavocytochrome b_{558} include incorporation of heme as a prerequisite for heterodimer assembly. *J. Biol. Chem*. 275:13986-93, 2000.

DeLeo, F.R., Allen, L.A., Apicella, M., and Nauseef, W.M. NADPH oxidase activation and assembly during phagocytosis. *J. Immunol*. 163:6732-40, 1999.

DeLeo, F.R., Renee, J., McCormick, S., Nakamura, M., Apicella, M., Weiss, J.P., and Nauseef, W.M. Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J. Clin. Invest*. 101:455-63, 1998.

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Dr. Hinnebusch received his Ph.D. in microbiology in 1991 from the University of Texas Health Science Center, San Antonio, studying the molecular structure and replication of linear plasmids of *Borrelia burgdorferi*, the arthropod-borne bacterial agent of Lyme disease.

Description of Research Program

Plague, caused by the Gram-negative bacterium *Yersinia pestis*, is a zoonosis that is present in wild rodent populations worldwide and is transmitted primarily by fleas. We use genome-scale methods to study the molecular mechanisms of *Y. pestis* host-parasite interactions, including the mechanisms that enable transmission by the principal vector of plague, the rat flea *Xenopsylla cheopis*. Animal model systems for both the insect vector and mammalian host are used to determine the biological function of *Y. pestis* genes that mediate transmission and pathogenicity. New targets for plague vaccine design and diagnostics are being sought by identifying antigens and virulence factors expressed by *Y. pestis* as it exits the flea and enters the mammal, and by characterizing the changes in pathogen and host gene expression induced upon entry into the mammal. Detailed understanding of how *Y. pestis* infects the flea vector may also lead to novel strategies to interrupt the transmission cycle, and may be applicable to other arthropod-borne agents.

Major Areas of Research

- Arthropod-borne bacterial zoonoses
- Molecular mechanisms of *Yersinia pestis* transmission by rat fleas
- Molecular mechanisms of bubonic plague pathogenesis

Selected Recent Publications

Hinnebusch, J., Cherepanov, P., Du, Y., Rudolph, A., Dixon, J.D., Schwan, T., Forsberg, A. Murine toxin of *Yersinia pestis* shows phospholipase D activity but is not required for virulence in mice. *Int. J. Med. Microbiol.* 290: 483-7, 2000.

Hinnebusch, B.J., Fischer, E.R., and Schwan, T.G. Evaluation of the role of the *Yersinia pestis* plasminogen activator and other plasmid-encoded factors in temperature-dependent blockage of the flea. *J. Infect. Dis.* 178: 1406-1415, 1998.

Hinnebusch, B.J. Bubonic plague: a molecular genetic case history of the emergence of an infectious disease. *J. Mol. Medicine* 75: 645-652, 1997.

Hinnebusch, B. J., Perry, R.D., and Schwan, T.G. Role of the Yersinia pestis hemin storage (hms) locus in the transmission of plague by fleas. *Science* 273: 367-370, 1996.

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M.Sc., 1993, University of Tuebingen, Germany (Biochemistry)
Ph.D., 1998, University of Tuebingen, Germany (Microbiology)

Description of Research Program

We are investigating the molecular basis of *Staphylococcus epidermidis* pathogenicity. *S. epidermidis* is a gram-positive opportunistic pathogen and the leading cause of nosocomial infections in the United States and worldwide. Its major pathogenic potential resides in the ability to form biofilms on indwelling medical devices. Research is focused on the expression of virulence factors by *S. epidermidis* under different circumstances, such as in a biofilm, on the regulation of these virulence factors, on bacterial cell-cell communication, and on the interaction of *S. epidermidis* with the host's immune system.

Major Areas of Research

- Global regulatory systems of *S. epidermidis*
- Peptide pheromone biosynthesis
- Staphylococcal biofilms

Selected Recent Publications

Otto, M., Sussmuth, R., Jung, G., and Gotz, F. Structure of the pheromone peptide of the *Staphylococcus epidermidis agr* system. *FEBS Lett.* 424: 89-94, 1998.

Otto, M., Sussmuth, R., Vuong, C., Jung, G., and Got, F. Inhibition of virulence factor expression in *Staphylococcus aureus* by the *Staphylococcus epidermidis agr* pheromone and derivatives. *FEBS Lett.* 450: 257-62, 1999.

Vuong, C., Gotz, F., and Otto, M. Construction and characterization of an *agr* deletion mutant of *Staphylococcus epidermidis. Infect. Immun.* 68: 1048-53, 2000.

Vuong, C., Saenz, H.L., Gotz, F., and Otto, M. Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus. J. Infect. Dis.* 182: 1688-93, 2000.

Otto, M., Echner, H., Voelter, W., and Gotz, F. Pheromone cross-inhibition between *Staphylococcus aureus* and *Staphylococcus epidermidis. Infect. Immun.* 69: 1957-60, 2001.

Patricia A. Rosa, Ph.D.

Senior Investigator, LHBP

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Dr. Rosa received her doctorate in 1980 from the Institute of Molecular Biology at the University of Oregon, studying cell type specific processing of a pituitary hormone. Dr. Rosa joined NIAID's Rocky Mountain Laboratories in 1988, where she is currently a Senior Investigator in the Laboratory of Human Bacterial Pathogenesis. She serves on the editorial board of *Research in Microbiology.*

Description of Research Program

Research in Dr. Rosa's laboratory focuses on *Borrelia burgdorferi*, the tick-transmitted bacterium that causes Lyme disease. The broad objective of research in her laboratory is to use a molecular genetic approach to elucidate the mechanisms of adaptation and variation in *B. burgdorferi.* Current and projected studies are designed to test the roles of specific genes and their products in the infectious cycle, and to identify additional genes that allow the spirochete to adapt, persist and be transmitted between ticks and mammals. Another focus of research in the lab is the unusual genome of *B. burgdorferi*, which comprises a small linear chromosome and a large number of circular and linear plasmids. How these DNA molecules replicate, why the genome is segmented, and the functional significance of this unique structure are topics of investigation. An ongoing endeavor in the laboratory is the development of a set of basic genetic tools for *B. burgdorferi.* The ability to perform routine genetic manipulations in *B. burgdorferi* is required to test the contributions of specific genes to the pathogenesis of Lyme disease and to assess their roles in the infectious cycle.

Major Areas of Research

- Vector/host-specific expression of outer surface protein genes
- Global and gene-specific regulatory mechanisms in *B. burgdorferi*
- Plasmid structure and function in *Borrelia*
- Genetic system in *B. burgdorferi*

Selected Recent Publications

Bono, J., Elias, A., Kupko, J., Stevenson, B., Tilly, K., and Rosa, P. Efficient targeted mutagenesis in *Borrelia burgdorferi. J. Bacteriol.* 182: 2445-2452, 2000.

Elias, A., Bono, J., Carroll, J., Stewart, P., Tilly, K., and Rosa, P. Altered stationary phase response in a *Borrelia burgdorferi rpoS* mutant. *J. Bacteriol.* 182: 2909-2918, 2000.

Stewart, P.E., Thalken, R., Bono, J. and Rosa, P. Isolation of a circular plasmid region sufficient for autonomous replication and transformation of infectious *Borrelia burgdorferi. Mol. Microbiol.* 39: 714-721, 2001.

Chaconas, G., Stewart, P. E., Tilly, K., Bono, J. L. and Rosa, P. Telomere resolution in the Lyme disease spirochete. *EMBO J.* 20: 3229-37, 2001.

Thomas G. Schwan, Ph.D.

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Dr. Schwan received his Ph.D. in 1983 in parasitology from the University of California at Berkeley, studying the ecology of fleas and plague in Lake Nakuru National Park, Kenya. From 1983 to 1986, he was a postdoctoral fellow at the Yale Arbovirus Research Unit, Yale University School of Medicine, studying tick-borne viruses. He joined the Rocky Mountain Laboratories in 1986. He served on the Editorial Board of the *Journal of Clinical Microbiology* for 9 years and currently is on the Editorial Board of *Vector Borne and Zoonotic Diseases.*

Description of Research Program

The Pathogen-Vector Molecular Interactions Section investigates bacterial pathogens transmitted by blood-feeding arthropods. Emphasis is directed toward the Lyme disease spirochete, *Borrelia burgdorferi*, and a relapsing fever spirochete, *Borrelia hermsii*, in their respective tick vectors. Other spirochetes and the causative agent of plague, *Yersinia pestis*, are also studied. Live colonies of ticks and fleas allow for studies to elucidate factors important for the infection of these bacteria in arthropods and for their biological transmission when ticks and fleas feed. Understanding bacterial adaptations associated with transmission are linked to developing better prevention and diagnostic strategies.

Major Areas of Research

- Adaptations of *Borrelia* spirochetes in ticks
- Genetic diversity of Lyme disease and relapsing fever spirochetes
- Developing better serological tests for human spirochetal infection
- Differential expression of *Yersinia pestis* genes using microarray

Selected Recent Publications

Schwan, T. G., Piesman, J., Golde, W. T., Dolan, M. C., and Rosa, P. A. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc. Natl. Acad. Sci. USA* 92: 2909 2913, 1995.

Schwan, T. G., and Hinnebusch, B. J. Bloodstream- versus tick-associated variants of a relapsing fever bacterium. *Science* 280:1938-1940, 1998.

Schwan, T. G., and Piesman, J. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. *J. Clin. Microbiol.* 38:382-388, 2000.

Porcella, S. F., Raffel, S. J., Schrumpf, M. E., Schriefer, M. E., Dennis, D. T., and Schwan, T. G. Serodiagnosis of louse-borne relapsing fever with glycerophosphodiester phosphodiesterase (GlpQ) of *Borrelia recurrentis*. *J. Clin. Microbiol.* 38:3561-1571, 2000.

Porcella, S.F., and Schwan, T.G. *Borrelia burgdorferi* and *Treponema pallidum:* a comparison of functional genomics, environmental adaptations, and pathogenic mechanisms. *J. Clin. Invest.* 107:651-656, 2001.

Laboratory of Immunogenetics

Susan K. Pierce, Ph.D., Chief

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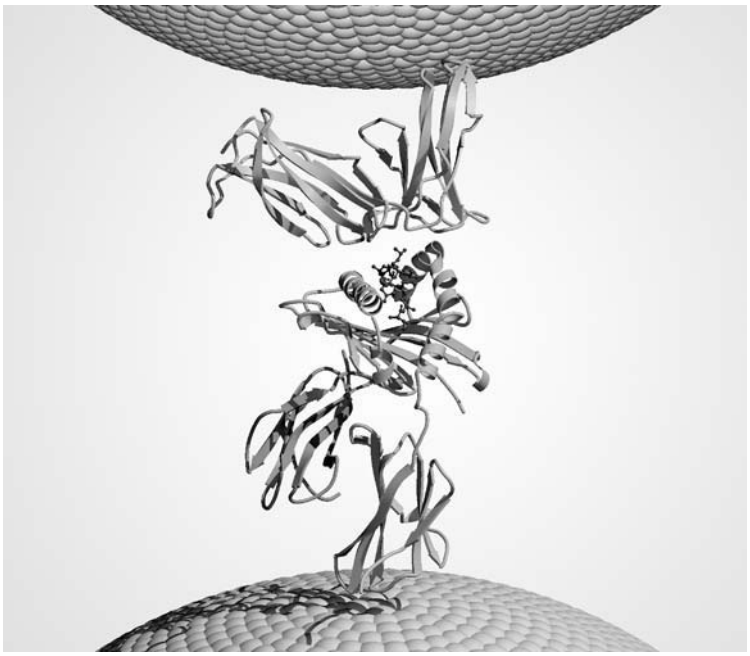

Sections and Units

Office of the Chief
Susan K. Pierce, Ph.D.

Chemotaxis Signaling Section
Tian Jin, Ph.D., Head

Lymphocyte Activation Section
Susan K. Pierce, Ph.D., Head

Molecular and Cellular Immunology Section
Eric O. Long, Ph.D., Head

Structural Biology Section
David N. Garboczi, Ph.D., Head

Structural Immunology Section
Peter D. Sun, Ph.D., Head

Autoimmunity and Functional Genomics Section
Silvia Bolland, Ph.D., Head

Tuberculosis Research Section
Clifton E. Barry, III, Ph.D., Head

Research Activities

The research in the Laboratory of Immunogenetics is focused on the cellular and molecular mechanisms that underlie the signaling functions of immune cell receptors. Research in the Laboratory encompasses a wide spectrum of experimental approaches from the structural determination of immune receptors to live cell image analysis of the behavior of chemotactic receptors. The laboratory members are highly interactive, creating a unique environment in which structural biology, molecular biology and cell biology are interfaced. The LIG is composed of seven independent research sections in addition to the Office of the Chief. The research in these sections focuses on a number of important immune cell signaling receptors and the outcome of receptor ligand engagement. Research includes investigation into: the structural basis of the recognition of antigen by gamma delta T cells; the structure and function of the natural killer (NK) cell inhibitory and activating receptors that allow NK cells to mount immune responses to cancer cells and pathogen-infected cells while sparing healthy cells; the molecular mechanisms underlying the function of the FcγRIIB in inhibiting immune responses and its role in autoimmunity; the signal transduction pathway in chemotaxis mediated by G protein coupled receptors; and the function of the B-cell antigen receptor in initiating signaling cascades and transporting antigen for processing with the MHC class II molecules. Additional studies are aimed at elucidating the structures of components of important pathogens and the cellular receptors with which they interact including the matrix protein of the Marburg virus, gamete and circumsporozoite proteins of the malarial parasite *Plasmodium sp.* and the dendritic cell surface HIV receptor, DC-SIGN. Interactions within the LIG are facilitated by weekly work-in-progress presentations detailing recent advances and future directions of the LIG fellows and students.

Susan K. Pierce, Ph.D.

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Dr. Pierce received her M.S. degree in microbiology from Yale University in 1974 and her Ph.D. in immunology from the University of Pennsylvania in 1976. After brief postdoctoral training at the University of Pennsylvania, Dr. Pierce joined the faculty in the Department of Biochemistry, Cell and Molecular Biology in the College of Arts and Sciences at Northwestern University in 1977. She was promoted to Professor in 1987 and served as chairman of her Department from 1990 to 1993. In 1996, Dr. Pierce was named to the William A. and Gayle Cook Chair in the Biological Sciences. Dr. Pierce was recruited to the NIH in 1999 to serve as Chief of the Laboratory of Immunogenetics.

Description of Research Program

The long standing research interests of my laboratory are in the molecular mechanisms by which B lymphocytes are activated by the binding of T-cell-dependent antigens to the clonally distributed B-cell antigen receptor (BCR). Antigen binding to the BCR triggers both the initiation of signaling cascades and the internalization of the BCR and bound antigen into the cell. The internalized antigen is transported to intracellular compartments where the antigen is processed for presentation with the MHC class II molecules to helper T cells. Using subcellular fractionation, electron microscopy and chemical crosslinking techniques, we described the intracellular compartments in which peptide class II complexes are assembled and the pathway by which the BCR delivers antigen to this compartment. An important conclusion drawn from these studies is that the signaling function of the BCR regulates the antigen trafficking function. Recent studies revealed how these two functions may be coordinated showing that BCR signaling and antigen targeting are initiated in cholesterol and sphingolipid-rich membrane microdomains termed lipid rafts. The rafts concentrate signaling and cytoskeleton components and appear to serve as platforms for both BCR signaling and trafficking. An exciting theme that emerged from the studies of rafts in B cells is that the access of the BCR to rafts is regulated by a variety of factors that function to regulate B-cell activation, including the developmental stage of the cell, the function of coreceptors and viral infection. Our goals are to understand the biochemical composition of rafts and the mechanism by which the BCR becomes associated with rafts following ligand binding. These studies are carried out with a view towards understanding how association with rafts can be regulated to enhance BCR signaling in the design of vaccines or dampen signaling in the therapies for autoimmunity.

Major Areas of Research

- Class II antigen processing
- BCR signaling
- Membrane microdomains

Selected Recent Publications

Cheng, P.C., Dykstra, M.L., Mitchell, R.N., and Pierce, S.K. A role for lipid rafts in B cell antigen receptor signaling and antigen targeting. *J. Exp. Med.* 290:1549, 1999.

Sproul, T.W., Malapati, S., Kim, J., and Pierce, S.K. B cell antigen receptor signaling occurs outside lipid rafts in immature B cells. *J. Immunol.* 165:6020, 2000.

Dykstra, M.L., Longnecker, R. and Pierce, S.K. Epstein Barr virus co-opts lipid rafts to block signaling and antigen transport function of the BCR. *Immunity* 14:57, 2001.

Cherukuri, A., Cheng, P.C., Sohn, H.W., and Pierce, S.K. The CD19/CD21 complex functions to prolong B cell antigen receptor signaling from lipid rafts. *Immunity* 14:169, 2001.

Clifton E. Barry, III, Ph.D.

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Dr. Barry received his Ph.D. in 1989 from Cornell University in organic and bioorganic chemistry studying the biosynthesis of complex natural products. Following postdoctoral research at Johns Hopkins University, Dr. Barry joined NIAID's research facility at the Rocky Mountain Laboratories (RML) in Hamilton, Montana. In 1998 he was tenured as Head of the Tuberculosis Research Section (TBRS). Dr. Barry is very active in the international community in activities that relate to the development of new chemotherapies for tuberculosis, serving as an advisor to the World Health Organization and the Global Alliance for TB Drug Development. Dr. Barry is a member of several editorial boards including the journals *Tuberculosis* and the *Journal of Bacteriology* and has authored more than 40 research publications in tuberculosis since entering the field 8 years ago.

Description of Research Program

The TBRS is focussed on basic and translational research with direct application to the global tuberculosis epidemic. Major projects currently include: understanding mechanisms of cell wall construction; understanding mechanisms of drug action and resistance using genomics for the front-line antituberculars; characterizing lipid and polyketide mediators of the host-pathogen interaction; characterizing the interaction of recent clinical isolates with the human immune system; synthesis and evaluation of analogs of second-line chemotherapeutic agents (in collaboration with Sequella, Inc.); structure-based drug design for enzymes involved in cell wall construction (in collaboration with GlaxoSmithKline and St. Jude's); development of animal models for TB chemotherapy; development and clinical evaluation of aerosol formulations for TB drug delivery; and advanced diagnostics development. TBRS has many active collaborations with academic and industrial investigators in North America, South Africa, Asia, Australia, and Europe that span basic and clinical research relating to the treatment of TB.

Major Areas of Research

- Tuberculosis drug discovery and drug mechanism of action
- Mechanisms of TB pathogenesis and the interaction of bacterial lipids with the host
- Preclinical and clinical evaluation of novel antituberculars and formulations
- Advanced diagnostics solutions for tuberculosis

Selected Recent Publications

DeVoss, J.J., Rutter, K., Zhu, Y., Schroeder, B.G., and Barry, C.E., 3rd. The salicylate-derived mycobactin siderophores of *Mycobacterium tuberculosis* are essential for growth in macrophages. *Proc. Natl. Acad. Sci. USA* 97: 1252, 2000.

Barry, C.E., 3rd, Schroeder, B.G. DNA microarrays: translational tools for understanding the biology of *Mycobacterium tuberculosis. Trends Microbiol.* 8: 209, 2000.

Stover, C.K., Warrener, P., VanDevanter, D.R., Arain, T.M., Langhorne, M.H., Anderson, S.W., Towell, A., Yuan, Y., Sherman, D.R., McMurray, D.N., Kreiswirth, B.N., Daniels, L., Barry, C.E., 3rd, Baker, W.R. A nitroimidazopyran drug candidate for the treatment of active and latent tuberculosis. *Nature* 405: 962-966, 2000.

Sampson, A.E., Mdluli, K., Bekker, L.G., Bosman, M., Barry, C.E., 3rd. Ethionamide activation and sensitivity in multidrug-resistant *Mycobacterium tuberculosis. Proc. Natl. Acad. Sci. USA* 97: 9677-82, 2000.

Primm, T.P., Anderson, S.J., Averbach, D., Rubin, H., Mizrahi, V., Barry, C.E., 3rd. The stringent response of *Mycobacterium tuberculosis* is required for long-term survival. *J. Bacteriol.* 182: 4889-98, 2000.

Manca, C., Tsenova, L. Bergtold, A., Freeman, S., Tovey, M., Musser, J.M., Barry, C.E., 3rd, Freedman, V.H., Kaplan, G. Virulence of a *Mycobacterium tuberculosis* clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of INF-α/β. *Proc. Natl. Acad. Sci. USA* 98: 5752-5757, 2001.

Silvia Bolland, Ph.D.

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Dr. Bolland received her Ph.D. in Molecular Biology from the University of Cantabria, Spain in 1991. After completing her postdoctoral training at Harvard and the Rockefeller University, she joined the Laboratory of Immunogenetics at NIH in 2001. She is the recipient of a S.L.E. Foundation Career Development Award and a Novel Research Grant Award from the Lupus Research Institute.

Description of Research

The Research Unit is interested in the regulation of the immune response by inhibitory signaling pathways and its relevance in the development of autoimmunity. Generally, autoimmune disease susceptibility is inherited in multigenic form and very little is known about genetic modifiers of disease. In order to identify these genetic determinants, congenic mouse lines are being generated and tested for differential gene expression by microarray analysis. Once candidate genes are identified, their ability to suppress autoimmune phenotypes in transgenic mice will be examined. These newly discovered genes could uncover potential routes for modifying ongoing disease in lupus or other autoimmune diseases. A central element in this genetic analysis is a new model for autoimmune disease: a mouse deficient in FcγRIIB, an IgG-binding receptor that inhibits antibody production and inflammatory responses. FcγRIIB function involves the association with the inositol phosphatase SHIP and prevents membrane localization of several PH domain-containing factors. Absence of SHIP results in reduced viability of the mice. To delineate the *in vivo* function of SHIP in specific cells while avoiding pleiotropic interactions, tissue-specific or inducible SHIP mutations will be characterized. Other inhibitory molecules will be tested similarly, including new genes from the mouse genome sequence that are predicted to encode inhibitory molecules.

Major Areas of Research

- Inhibitory signaling pathways
- Autoimmunity

Selected Recent Publications

Ravetch, J.V. and Bolland, S. IgG Fc receptors. *Ann. Rev. Immunol.* 19: 275-90, 2001.

Bolland, S. and Ravetch, J.V. Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. *Immunity* 13: 277-85, 2000.

Bolland, S. and Ravetch, J.V. Inhibitory pathways triggered by ITIM-containing receptors. *Adv. Immunology* 72:149-77, 1999.

Bolland. S., Pearse, R.N., Kurosaki, T., and Ravetch, J.V. SHIP modulates immune receptor responses by regulating membrane association of Btk. *Immunity* 8: 509-516, 1998.

Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J.V. Deletion of SHIP or SHP-1 reveals two distinct pathways for inhibitory signaling. *Cell* 90:293-301, 1997.

Ono, M., Bolland, S., Tempst, P. and Ravetch, J.V. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc-gamma-RIIB. *Nature* 383: 263-266, 1996.

David N. Garboczi, Ph.D.

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Dr. Garboczi obtained his Ph.D. from the University of Oregon after performing his predoctoral research at the Johns Hopkins School of Medicine in the biochemistry of the ATP synthase of mammalian mitochondria. Following postdoctoral research in molecular immunology at Harvard University, he joined the Structural Biology Section in 1997 as a tenure-track Investigator.

Description of Research Program

T-Cell Receptors

Members of the Structural Biology Section are studying T-cell antigen receptors (TCR) to more fully understand the requirements for T-cell activation in an immune response. The two broad classes of TCR, alpha/beta TCR and gamma/delta TCR, are under investigation. The T-cell coreceptors CD8 and the subunits of CD3 are also being studied. The goal is to analyze their proteins both biochemically and structurally. To do so requires production of sufficient amounts of protein for study and characterization of the appropriate function of the recombinant proteins. Further study of their binding to ligands and of their 3-dimensional structure by x-ray crystallography should lead to insights into how these proteins function in the immune system.

Proteins from Viruses and Parasites

Researchers are working with proteins from hepatitis and filoviruses, addressing questions of virus structure and organization. The Section is pursuing the crystallization of several proteins involved in the pathogenesis caused by the malarial parasite and under study as potential vaccine candidates.

Membrane Proteins

About 30 percent of all proteins can be found in a cellular membrane. However, in contrast to the many structures of known soluble proteins, only a handful of membrane protein structures have been determined. This Section is working to crystallize and determine the structure of a membrane protein by x-ray crystallography. The aim is to produce sufficient amounts of and to crystallize several membrane proteins. The approach is to produce the proteins in bacteria as the denatured polypeptide chains that bacteria often produce and then to renature the proteins to their native and functional conformations. After obtaining sufficient amounts of the pure protein, Section scientists will crystallize the protein in preparation for the x-ray crystallographic structure determination.

Major Areas of Research

- T-cell receptors
- Proteins from viruses and parasites
- Membrane proteins

Selected Recent Publications

Allison, T.J., Winter, C.C., Fournie, J. -J., Bonneville, M., and Garboczi, D.N. Structure of a human gamma delta T-cell antigen receptor. *Nature* 411: 820-824, 2001.

Garboczi, D.N., and Biddison, W.E. Shapes of MHC restriction. *Immunity* 10: 1-7, 1999.

Garboczi, D.N., Ghosh, P., Utz, U., Fan, Q.R., Biddison, W.E., and Wiley, D.C. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384: 134-141, 1996.

Tian Jin, Ph.D.

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Dr. Jin received his B.S. in biology from the Beijing University, China in 1984 and his Ph.D. degree from the Department of Biochemistry at the Robert Wood Johnson Medical School at Rutgers-UMDNJ in 1994. From 1994 to 2000, he was a postdoctoral fellow in the Department of Biological Chemistry at Johns Hopkins University School of Medicine. Dr. Jin was appointed Instructor in the Department of Cell Biology and Anatomy at Johns Hopkins University School of Medicine in 2001. In July 2001, Dr. Jin joined the Laboratory of Immunogenetics as a tenure-track Investigator.

Description of Research Program

Our research goal is to understand the molecular mechanisms of chemotaxis-the directional motility of cells in gradients of chemoattractants. Chemokines are sensed by G protein-coupled receptors (GPRC) and transduced through downstream signaling proteins. Immune cells such as neutrophils, macrophages, B cells and T cells, as well as the social amoeba, *Dictyostelium discoideum*, employ evolutionary conserved signaling mechanisms to mediate their chemotactic movement. Using a combination of genetic, biochemical and cell biological approaches, we employ the model system *D. discoideum* to identify components and to decipher signal transduction pathways involved in chemotactic sensing and responses. Currently, our research focuses on two negative regulators of G protein signaling: a newly discovered *mirA* and a RGS protein. We will apply information learned from *D. discoideum* to immune cells. One current project is to establish a system to visualize activation of GPCRs in living cells. Activation of a chemoattractant receptor recruits a PH-GFP, a pleckstrin homology domain tagged with a green fluorescence protein, from cytoplasm to cell membrane. We are expressing GFP-tagged signaling proteins in cell lines and in transgenic mice and are using a time-lapse fluorescence microscope to monitor dynamic distributions of these proteins during signaling events.

Major Areas of Research

- Chemotaxis
- G protein-coupled receptors

Selected Recent Publications

Jin, T., Soede, R.M., Liu, J., Kimmel, A.R., Devreotes, P., and Schaap, P. Temperature sensitive Gβ mutants discriminate between G protein dependent and G protein independent signaling mediated by serpentine receptors. *EMBO J.* 17: 5076-5084, 1998.

Jin, T., Amzel, M., Devreotes, P. and Wu, L. Selection of Gβ subunits with point mutations that fail to activate specific signaling pathways in vivo: dissecting cellular responses mediated by a heterotrimeric G protein in *D. discoideum*. *Mol. Biol. Cell* 9: 2949-2961, 1998.

Jin, T., Zhang, N., Yu, L., Parent, C. and Devreotes, P. Localization of the G protein βγ complex in living cells during chemotaxis. *Science* 287: 1034-1036, 2000.

Janetopoulos, C., Jin, T., and Devreotes, P. Receptor mediated activation of heterotrimeric G-proteins in living cells. *Science* 291: 2408-2411, 2001.

Eric O. Long, Ph.D.

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Dr. Long received his Ph.D. from the University of Geneva, Switzerland in 1976. After completing a postdoctoral fellowship at the National Cancer Institute from 1977 to 1980, Dr. Long joined the faculty of the University of Geneva as a junior member. In 1983 he was recruited to the NIH as a tenure-track Investigator in the Laboratory of Immunogenetics. He became Section Head in 1988 and was elected to the Senior Biomedical Research Service in 1998. Dr. Long is a recipient of the NIH Director's Award and is a member of the Advisory Board for the International Histocompatibility Working Group.

Description of Research Program

While sparing normal cells from lysis, natural killer (NK) cells provide an effective immune defense against tumor cells, viruses, and other intracellular pathogens. NK cell cytotoxicity is kept under control by inhibitory receptors that recognize MHC class I molecules on target cells and deliver a dominant inhibitory signal. NK cell cytotoxicity can be induced when target cells fail to express MHC class I ligands of inhibitory receptors, as may occur in tumor cells or virus-infected cells. The function of inhibitory receptors is mediated by the specific recruitment and activation of the tyrosine phosphatase SHP-1 through a cytoplasmic immunoreceptor tyrosine-based inhibition motif (ITIM). The definition of the ITIM by Dr. Long's group has led to the identification of several other receptors with inhibitory function that are expressed on various types of cells. Many effector cell types that have important roles in the innate immune responses are under the type of negative control that was previously associated with regulation of NK cell function. Current specific aims are (1) to determine what receptor-ligand interactions are necessary to induce target cell killing by NK cells; (2) to define precisely how inhibitory receptors block activation signals; and (3) to determine the specific requirements for activation of resting NK cells to produce interferon-gamma, to adhere to target cells, and to become cytotoxic.

Major Areas of Research

- Natural killer (NK) cell receptors
- Regulation through inhibitory receptors
- Signaling pathways in NK cell activation

Selected Recent Publications

Rajagopalan, S., and Long, E.O. An HLA-G-specific receptor expressed on all natural killer cells. *J. Exp. Med.* 189: 1093-1099, 1999.

Long, E.O. Regulation of immune responses through inhibitory receptors. *Annual Rev. Immunol.* 17: 875-904, 1999.

Watzl, C., and Long, E.O. Exposing tumor cells to killer cell attack. *Nature Med.* 6: 867-868, 2000.

Burshtyn, D.N., Shin, J., Stebbins, C., and Long E.O. Adhesion to target cells is disrupted by the killer cell inhibitory receptor. *Curr. Biol*. 10: 777-780, 2000.

Rajagopalan, S., Fa, J. Long, E.O. Cutting edge: Induction of IFN-gamma production but not cytotoxicity by the killer cell Ig-like receptor kir2d14 (cd158d) in resting NK cells. *J. Immunol.* 167: 1877-81, 2001.

Peter D. Sun, Ph.D.

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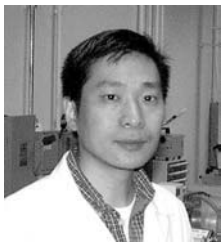

Dr. Sun obtained his Ph.D. from the Molecular Biology Institute, University of Oregon for the study of structure and thermostability of phage T4 lysozyme using X-ray crystallography. He then joined NIDDK for his postdoctoral training in 1991, focusing on the structure and function of cytokines. In particular, he determined the crystal structure of a human transforming growth factor, TGF-beta 2. He joined NIAID in 1994.

Description of Research Program

My research focus has been on the structure and function of immunoreceptors. In particular, we study how the receptors recognize their ligands and initiate activation or inhibitory signals. We primarily choose to use the X-ray crystallography technique to define three-dimensional molecular structures of receptors and their ligand complexes. By doing so, we can visualize the receptor-ligand molecular interface and thus map out the hot spots on the receptor, the regions critical for ligand recognition. Knowing the shape of a receptor-ligand interface and the residues involved will help us to diagnose the diseases caused by the impairment of receptor function and to design potential new drugs that can inhibit the function of these receptors.

Recent advances in the molecular and cellular biology of innate immune functions have opened a new, exciting frontier for structural biology—to reveal the receptor activation mechanism involved in innate immunity. At the center of our interest is the mechanism by which NK cells distinguish between self and non-self and, thereby, direct their cytolytic killing against virally infected rather than healthy host cells. Two families of inhibitory receptors, the killer immunoglobulin receptors (KIR) and the C-type lectin-like receptors, have been identified on human NK cells to interact with class I MHC antigens and, thus, may be critical in mediating this self versus non-self recognition. We have determined the crystal structures of an HLA-Cw3 recognizing KIR and its complex with the HLA molecule. This is the first structure of a KIR/HLA complex, and it illustrates the contribution of the class I peptide to KIR recognition and the nature of allotypic specificity of the receptor.

Another receptor involved in NK cell-mediated innate immunity is an Fc receptor, FcγIII. Fcγ receptors are expressed on the surface of macrophages, mast cells, and NK cells to mediate opsonization of antibody-coated pathogens and antibody-directed cellular cytotoxicity. They are also implicated in certain autoantibody mediated autoimmune diseases. To define how immunoglobulins bind and activate humoral immune systems through their Fc receptors, we crystallized and solved the X-ray structure of FcγIII in complex with an Fc fragment of IgG1. We then explored using small molecular ligands that can inhibit the receptor function as potential therapeutic reagents. We have designed four peptides and demonstrated their ability to bind to receptor FcγRIII, opening new ways of designing therapeutic compounds.

Major Areas of Research

- Structural immunology
- The structure of immune synapses

Selected Recent Publications

Radaev, S., S.A. Motyka, W-H Fridman, C. Sautes-Fridman, and P.D. Sun. The structure of a human type III Fcgamma receptor in complex with Fc. *J. Biol. Chem.* 276:16469-16477, 2001.

Radaev, S., and P.D. Sun. Recognition of IgG by Fcgamma receptor: The role of Fc glycosylation and the binding of peptide inhibitors. *J. Biol. Chem.* 276:16478-16483, 2001.

Laboratory of Immunology

William E. Paul, M.D., Chief

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Laboratory Sections and Units

Office of the Chief
William E. Paul, M.D., Chief
Ronald N. Germain, M.D., Ph.D., Deputy Chief

Biochemistry and Immunopharmacology Section
Michail V. Sitkovsky, Ph.D., Head

Bioorganic Chemistry Section
John K. Inman, Ph.D., Head

Cellular Immunology Section
Ethan M. Shevach, M.D., Head

Lymphocyte Development Unit
Hua Gu, Ph.D., Head

Immune Regulation Unit
William E. Paul, M.D., Head

Lymphocyte Biology Section
Ronald N. Germain, M.D., Ph.D., Head

Molecular Biology Section
David H. Margulies, M.D., Ph.D., Head

Molecular Development of the Immune System Section
Michael J. Lenardo, M.D., Head

Molecular Immunogenetics Section
Rose G. Mage, Ph.D., Head

Research Activities

The major research activities of LI scientists concern the basic genetics, molecular biology, cell biology, and cellular immunology of the immune system. Important topics of interest are how dysregulation of the immune system results in autoimmune diseases and what strategies might be valuable for vaccine development. Specific areas of current investigation include:

- Early lymphocyte development
- T- and B-cell receptor gene rearrangement
- MHC molecule structure and function
- Antigen processing
- T cell and cytokine receptor signal transduction
- Apoptotic cell death
- Regulation of cytokine production and activity of cytokines
- Mechanisms of cytotoxicity
- Regulatory T cells and control of autoimmune responses.

William E. Paul, M.D.

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Former Director, Office of AIDS Research and Associate NIH Director for AIDS Research, NIH, (1994-1997). Editorial Boards: *Annual Review of Immunology*, *Immunity*, *Journal of Experimental Medicine*. Societies: Assn. of American Immunologists (President 1986-87); American Society for Clinical Investigation (President 1980-81); Assn. of American Physicians. Honors: National Academy of Sciences, USA (1982-present); Institute of Medicine (1990- present); American Academy of Arts and Sciences (1993-present); Founders Prize, Texas Instruments Fdn. (1979); 3M Life Sciences Award, FASEB (1988); D.Sc. (Honorary) State University of New York (1991); Raymond & Beverly Sackler Senior Professor by Special Appointment, Tel Aviv Univ. (2000-present).

Description of Research Program

My group's major interest is in studying the biology of CD4+ T cells. We are particularly interested in the early events that control IL-4 and IFNg production and that determine TH1/TH2 differentiation. To understand the function of TH2 cells, we analyze the role of accessibility in the regulation of expression of the *Il4* gene. We have obtained evidence that *Il4* is not simply in an "on/off" state but can exist in many stable forms with different degrees of accessibility, accounting for the phenomenon of IL-4 monoallelism. We have also studied the biology of memory CD4+ T cells and have shown that primed, resting TH1 cells are more subject than naïve T cells to clonal elimination and anergy when confronted with their cognate antigens *in vivo*, providing approaches to the control of ongoing immune responses. Using array analysis, we have identified a Stat6-inducible molecule, Gfi-1, that plays a major role in the expansion of TH2 cells but fails to influence the growth of TH1 or THnull cells. Our general goal is to obtain a cellular and molecular picture of lymphocyte differentiation and dynamics and to describe these in quantitative terms that can be used to understand *in vivo* immune responses. To that end, we have a major interest in modeling the behavior of naïve and memory CD4+ T cells undergoing both homeostatic and antigen-driven proliferation.

Major Areas of Research

- Cytokines: characterization, regulation of production, mode of action, mechanism of receptor function
- Regulation of lymphocyte activation, differentiation, and proliferation
- Immunologic memory and strategies for vaccine development

Selected Recent Publications

Grossman, Z., Polis, M., Feinberg, M.B., Grossman, Z., Levi, I., Jankelevich, S., Yarchoan, R., Boon, J., de Wolf, F., Lange, J.M.A., Goudsmit, J., Dimitrov, D.S., Paul, W.E. Ongoing HIV dissemination during HAART. *Nature Med.* 5: 1099-1104, 1999.

Zhu, J., Huang, H., Guo, L., Stonehouse, T., Watson, C. J., Hu-Li, J., and Paul, W. E. Transient inhibition of interleukin 4 signaling by T cell receptor ligation. *J. Exp. Med.* 192: 1125-1134, 2000.

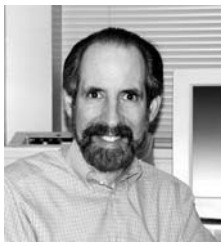
Noben-Trauth, N., Hu-Li, J., and Paul, W. E. Conventional naive CD4+ T cells provide an initial source of IL-4 during Th2 differentiation. *J. Immunol.* 165: 3620-3625, 2000.

Grossman. Z., and Paul, W.E. The impact of HIV on naive T-cell homeostasis. *Nature Med.* 6: 976-977, 2000.

Ben-Sasson, S. Z., Gerstel, R., Hu-Li, J., and Paul, W. E. Cell division is not a "clock" measuring acquisition of competence to produce IFN-gamma or IL-4. *J. Immunol.* 166: 112-120, 2001.

Hu-Li, J., Pannetier, C., Guo, L., Löhning, M., Gu, H., Watson, C., Assenmacher, M., Radbruch, A., and Paul, W.E. Regulation of expression of IL-4 alleles: analysis using a chimeric GFP/IL-4 gene. *Immunity* 14:1-11, 2001.

Guo, L. Hu-Li, J., Zhu, J., Pannetier, C., Watson, C., McKenzie, G.J., McKenzie, A.N.J. and Paul, W.E. Disrupting Il13 impairs production of IL-4 specified by the linked allele. *Nature Immunol.* 2: 461-466, 2001.



Ronald N. Germain, M.D., Ph.D.

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Head, Lymphocyte Biology Section, LI

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1970–B.S. and M.S., Brown University; 1976–M.D. and Ph.D., Harvard Medical School and Harvard University; 1976-1982–Instructor, Assistant, and Associate Professor, Pathology, Harvard Medical School; 1982-1987–Senior Investigator, Laboratory of Immunology, NIAID, NIH; 1987-Date–Chief, Lymphocyte Biology Section, Laboratory of Immunology, NIAID, NIH; 1994-present–Deputy Chief, Laboratory of Immunology, NIAID, NIH.

Description of Research Program

The Lymphocyte Biology Section (LBS) studies basic aspects of how white blood cells called T lymphocytes recognize and respond to foreign substances known as antigens. A current focus of the LBS is on the consequences of T-cell receptor recognition of peptide-MHC molecule antigen complexes, especially how binding events are translated into intracellular signals that regulate T-cell differentiation. Previous accomplishments of the LBS in this area are the description of TCR antagonists and partial agonists and the discovery that such ligands induce a distinct set of early intracellular tyrosine phosphorylation events. Ongoing work centers on understanding the differences in intracellular signals generated by agonist versus partial agonists/antagonists and the molecular interactions that account for normal and altered signaling. These studies are being extended to an analysis of the role of self-recognition in responses to foreign antigens, visualization of protein movement at the interface of T cells and antigen bearing cells, kinetic measurements of the interaction of various proteins with the TCR on living T cells, and development of computer models of how ligand recognition is translated into intracellular signals controlling T-cell function. We are continuing to study T-cell development in the thymus. We have developed a new model system for examining the role of antigen specific and unspecific signals in T-cell lineage (CD4 versus CD8) choice, as well as the nature of TCR signaling leading to effective positive and negative selection.

Finally, we have developed confocal microscopy methods that permit the direct visualization of the interactions of T cells and dendritic cells in intact lymph nodes. This method is being used to explore how T cells migrate to the sites of antigen display, the duration of cell-cell interactions, and the movement of specific protein molecules during cell locomotion and cell-cell contact. We plan to use FRET methods and signaling sensitive indicator molecules to visualize specific protein-protein interactions and signal transduction during an ongoing immune response.

Major Areas of Research

- T-cell receptor signaling in response to peptide / MHC molecule binding
- Thymic development
- Generation of peptide / MHC ligands and the interaction of T cells with antigen presenting cells

Selected Recent Publications

Castellino, F., Boucher, P.E., Eichelberg, K., Mayhew, M., Rothman, J.E., Houghton, A.N., and Germain, R.N. Receptor-mediated uptake of antigen/heat shock protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. *J. Exp. Med.* 191: 1957-1964, 2000.

Yasutomo, K., Doyle, C., Miele, L., Fuchs, C., and Germain, R.N. The duration of antigen receptor signaling determines CD4+ versus CD8+ T-cell lineage fate. *Nature* 404: 506-510, 2000.

Dorfman, J.R., Stefanova, I., Yasutomo, K., and Germain, R.N. CD4+ T cell survival is not directly linked to self-MHC-induced TCR signaling. *Nature Immunol.* 1: 329-335, 2000.

Hua Gu, Ph.D.

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Dr. Gu obtained his Ph.D. from the University of Cologne, Germany, for the study of antibody repertoire and B-cell development in normal and genetic mutant mice. After completion of postdoctoral research at the same university, he joined the LI in 1994 and is currently the Head of the Lymphocyte Development Unit. His research focuses on intracellular signaling processes involved in lymphocyte development and function, with emphasis on understanding the relationship between dysregulation of lymphocyte activation signals and immune system diseases.

Description of Research Program

Balanced intracellular signaling process plays an essential role in the control of lymphocyte activation and immune tolerance induction. It is therefore important to understand the regulatory mechanisms responsible for maintaining the homeostasis of intracellular signaling activities. One of the research interests in this laboratory is to identify intracellular signaling components involved in the modulation of T-cell activation signals using various genetic, cellular, and molecular biological approaches. Current projects include generating and analyzing several mouse models deficient in the adaptor signaling molecules Cbl, Cbl-b, and Grb-2. Preliminary data indicate that both Cbl and Cbl-b participate in the regulation of T-cell activation signaling, and defect of these genes may lead to either the altered T-cell development or the hyperresponsiveness of T cells and autoimmune diseases.

Major Areas of Research

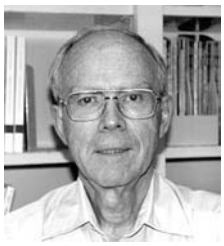
- Lymphocyte development and activation
- Intracellular signaling
- Autoimmunity

Selected Recent Publications

Hu-Li, J., Pannetier, C., Guo, L., Lohning, M., Gu, H., Watson, C., Assenmacher, M., Radbruch, A., Paul, W.E. Regulation of expression of IL-4 alleles: analysis using a chimeric GFP/IL-4 gene. *Immunity* 14: 1-11, 2001.

Chiang, Y.J., Kole, H.K., Brown, K., Naramura, M., Fukuhara, S., Hu, R.J., Jang, I.K., Gutkind, J.S., Shevach, E., Gu, H. Cbl-b regulates the CD28 dependence of T-cell activation. *Nature* 403: 216-20, 2000.

Harrison, K.A., Thaler, J., Pfaff, S.L., Gu, H., Kerhl, J.H. Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in Hlxb-9-deficient mice. *Nature Genet.* 23: 71-5, 1999.



John K. Inman, Ph.D.

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Dr. Inman received his Ph.D. in biochemistry from Harvard University. His posts then included Research Group Leader, Michigan Department of Health Labs; Director of the Division of Biochemistry, Ortho Pharmaceutical Corporation; Special U.S. Public Health Services Postdoctoral Fellow in the Department of Biophysics, Johns Hopkins University School of Medicine; and Senior Staff member of Laboratory of Immunology, NIAID from 1965 to the present.

Description of Research Program

The principal focus of research carried out in the Bioorganic Chemistry Section is the design and synthesis of novel, small molecular weight compounds that target and inactivate the nucleocapsid protein (NCp7) of the human immunodeficiency virus (HIV). The aim is to discover and develop drugs that will not easily fall prey to drug resistance owing to the highly conserved nature of NCp7 and its intolerance to mutations. Compounds designed and synthesized in Dr. Inman's lab are studied and evaluated for their biological activities (antiviral properties, toxicities, pharmacokinetics, etc.) in collaboration with other laboratories in NCI, NIAID and with Achillion Pharmaceutical Corporation of New Haven, CT (in a CRADA agreement). We have prepared and identified a number of active anti-HIV compounds that inactivate NCp7 through an initial covalent interaction of certain thioesters and related substances with zinc finger cysteine side chains of NCp7.

Additionally, this Section is involved in developing novel bioconjugate molecules that are specially designed as immunomodulators for basic collaborative studies on immune responses and as vaccine constructs. One study, in particular, has been carried out with a collaborating laboratory at New York University wherein our constructs are designed to modify the clustering of B-cell surface antigen receptors with several co-receptors involved in activation, apoptosis and differentiation of B cells when they are stimulated by antigen or antigen-complement complexes. We have also prepared conjugate vaccine constructs based on heat-killed *Brucella abortus* organisms as carrier and adjuvant-bearing peptide antigen epitopes from HIV-1. These constructs are designed to promote strong cytolytic T-cell and neutralizing antibody responses.

Major Areas of Research

- Developing anti-HIV drugs directed against a novel target
- Designing bioconjugates for studies on B-cell activation and differentiation
- Designing and preparing novel anti-HIV vaccine constructs

Selected Recent Publications

Turpin, J.A., Song, Y., Inman, J.K., Huang, M., Wallqvist, A., Maynard, A., Covell, D.G., Rice, W.G., and Appella, E. Synthesis and biological properties of novel pyridinioalkanoyl thiolesters (PATE) as anti-HIV-1 agents that target the viral nucleocapsid protein zinc fingers. *Journal of Medicinal Chemistry* 42: 67-86, 1999.

Mongini, P.K.A. and Inman, J.K. Cytokine dependency of human B cell cycle progression elicited by ligands which coengage BCR and the CD21/CD19/CD81 costimulatory complex. *Cellular Immunology* 207: 127-140, 2001.

Michael J. Lenardo, M.D.

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Dr. Lenardo graduated with a B.A. from Johns Hopkins University and an M.D. from Washington University in St. Louis. He performed clinical work in internal medicine and research at the University of Iowa and received postdoctoral training at the Whitehead Institute for Biomedical Research at MIT. He established an independent research unit in LI in 1989 and became a Senior Investigator and Section Head in 1994. Dr. Lenardo serves on several editorial boards and has given numerous lectures around the world on his work on the molecular regulation of immune homeostasis. His work focuses on lymphocyte apoptosis, autoimmunity, and HIV pathogenesis.

Description of Research Program

The Section investigates the molecular regulation of T lymphocytes, particularly as that regulation relates to immunological tolerance, apoptosis, and autoimmune diseases such as multiple sclerosis, myasthenia gravis, and similar diseases. Laboratory scientists use both molecular biology and cellular immunology techniques to pursue these investigations. The goal is to understand the pathogenesis of autoimmunity from the vantage point of T-cell regulation as well as develop novel means of immunomodulation of these diseases. Recently, the Section has undertaken the investigation of how HIV causes the death of CD4+ T lymphocytes. Such studies could lead to a better understanding of viral pathogenesis as well as the development of new treatments for, or vaccines against, AIDS.

Major Areas of Research

- Molecular regulation of immune homeostasis
- CD4+ T-cell depletion in HIV infection

Selected Recent Publications

Siegel, R.M., Lenardo, M.J. To B or not to B: TNF family signaling in lymphocytes. *Nature Immunol.* 2:577-8, 2001.

Straus, S.E., Jaffe, E.S., Puck, J.M., Dale, J.K., Elkon, K.B., Rosen-Wolff, A., Peters, A.M., Sneller, M.C., Hallahan, C.W., Wang, J., Fischer, R.E., Jackson, C.M., Lin, A.Y., Baumler, C., Siegert, E., Marx, A., Vaishnaw, A.K., Grodzicky, T., Fleisher, T.A., Lenardo, M.J. The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. *Blood* 98: 194-200, 2001.

Siegel, R.M., Chan, F.K., Chun, H.J., Lenardo, M.J. The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. *Nature Immunol.* 1: 469-74, 2000.

Locksley, R.M., Killeen, N., Lenardo, M.J. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104:487-501, 2001.

McFarland, H.I., Lobito, A.A., Johnson, M.M., Palardy, G.R., Yee, C.S., Jordan, E.K., Frank, J.A., Tresser, N., Genain, C.P., Mueller, J.P., Matis, L.A., Lenardo, M.J. Effective antigen-specific immunotherapy in the marmoset model of multiple sclerosis. *J. Immunol.* 166: 2116-21, 2001.

Siegel, R.M., Frederiksen, J.K., Zacharias, D.A., Chan, F.K., Johnson, M., Lynch, D., Tsien, R.Y., Lenardo, M.J. Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* 288: 354-7, 2000.

Chan, F.K., Chun, H.J., Zheng, L., Siegel, R.M., Bui, K.L., Lenardo, M.J. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. *Science* 288: 2351-4, 2000.

Hornung, F., Scala, G., Lenardo, M.J. TNF-alpha-induced secretion of C-C chemokines modulates C-C chemokine receptor 5 expression on peripheral blood lymphocytes. *J. Immunol.* 164: 6180-7, 2000.

Rose G. Mage, Ph.D.

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Dr. Mage obtained her Ph.D. from Columbia University for studies of the structure and size of the combining sites of antibodies for the polysaccharide antigens dextran and pneumococcal polysaccharide. She joined the Laboratory of Immunology in 1963, first as a postdoctoral fellow and then as a Staff Scientist. She has been Head of the Molecular Immunogenetics Section since 1988 and an Adjunct Professor of Genetics at George Washington University since 1976. Her research focuses on the regulated development and diversification of the antibody repertoire. She serves on numerous editorial boards, participates on intramural and extramural committees and boards, and was elected to the Senior Biomedical Research Service.

Description of Research Program

The goals of the Molecular Immunogenetics Section are to understand the development of the immune system with special emphasis on B lymphocytes that develop to produce protective antibodies in the blood and at mucosal surfaces in response to vaccines and infections. Mucosal surfaces such as those of the respiratory, digestive, and genitourinary tracts are important sites of invasion of infectious organisms ranging from those causing sexually transmitted diseases to tuberculosis, influenza, and cholera. Characterized are genes that code for antibody molecules as well as genes that are needed to regulate development of the cells producing these molecules. Scientists use tools of genetics and cell and molecular biology and the rabbit as an animal model. In the rabbit, the appendix is an important site of development of the cells destined to produce protective antibodies. In the young rabbit, there is rapid growth and expansion of these B lymphocytes, but there is also considerable cell death. Positive selection for growth and survival of developing cells occurs via signals directed in part through copies of the antibody molecules that appear on the surfaces of the developing cells. Scientists use techniques of hydraulic micromanipulation and laser capture microdissection to collect single cells for studies of clonal development of B lymphocytes. Diversity of DNA sequences is generated during clonal expansion by a mechanism similar to gene conversion found in yeast and other fungi. This mechanism allows one gene to be converted in part to resemble another. The rabbit uses this mechanism to make different antibody structures that can recognize a variety of different foreign antigens. A current goal of the research is to further understand the mechanism of gene conversion and whether it can contribute to affinity maturation during specific immune responses.

Major Areas of Research

- Immunogenetics
- B-cell development
- Diversification of antibody sequences

Selected Recent Publications

Schiaffella, E., Sehgal, D., Anderson, A.O., and Mage, R. G. Gene conversion and hypermutation during diversification of VH sequences in developing splenic germinal centers of immunized rabbits. *J. Immunol.* 162: 3984-3995, 1999.

Sehgal, D., Johnson, G., Wu, T. T., and Mage, R. G. Generation of the primary antibody repertoire in rabbits: Expression of a diverse set of *Igk*-V genes may compensate for limited combinatorial diversity at the heavy chain locus. *Immunogenetics* 50: 31-42, 1999.

Sehgal, D., Schiaffella, E., Anderson, A. O., and Mage, R. G. Generation of heterogeneous rabbit anti- DNP antibodies by gene conversion and hypermutation of rearranged VL and VH gene during clonal expansion of B cells in splenic germinal centers. *Eur. J. Immunology* 30: 3634-3644, 2000.

David H. Margulies, M.D., Ph.D.

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1978-1980, Resident, Medicine, Columbia/Presbyterian Med. Center, N.Y.
1980-1983, Research Associate, Laboratory of Molecular Genetics, NICHD
1983-1987, Investigator, LI, NIAID
1987-Date, Senior Investigator, LI, NIAID

Description of Research Program

The research program of the Molecular Biology Section (MBS) of the Laboratory of Immunology is directed toward a precise molecular understanding of critical interactions that initiate, control, and perpetuate the immune response. To this aim, this laboratory has focused on: 1) the genes and encoded proteins of the major histocompatibility complex (MHC), MHC-I, MHC-Ib, and MHC-II molecules, whose primary function is to present antigens to T lymphocytes, but which in some cases also serve as critical recognition elements for natural killer (NK) cells; 2) receptors on T lymphocytes, particularly T-cell receptors, that provide discriminatory recognition and initial signals in T-cell development and activation; and 3) receptors on natural killer (NK) cells that mediate both activating and inhibitory signals and thus the biological activity of these cells. The MBS exploits a wide variety of contemporary methodologies in efforts to understand these molecular and cellular events that determine both the normal immune response and, when dysregulated, contribute to autoimmune diseases and immunodeficiency. This laboratory has pioneered in the development of genetic engineering of MHC molecules, TCR, and NK receptors, and in the quantitative measurement of MHC/TCR, MHC/NK, and MHC/peptide interactions using biophysical methods such as surface plasmon resonance. In addition, we have determined crystallographic structures of a TCR domain and of an MHC I/NK receptor complex. We have performed a comprehensive analysis of the structure/function relationship of MHC I/NK cell interactions by mutagenesis and binding studies. Most recently, we have generated several animal models for autoimmune gastritis by making TCR transgenic mice with pathogenic TCR cloned from autoimmune T cells. Our long-term goal is to provide insight into molecular manipulation of the immune response based on detailed understanding of ligand/receptor interactions that are key to immune recognition.

Major Areas of Research

- Molecular and immune recognition
- Molecular structure and function
- Autoimmunity and control of T-cell and NK-cell activation and function

Selected Recent Publications

Tormo, J., Natarajan, K., Margulies, D.H., and Mariuzza, R.A. Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand. *Nature* 402: 623-631, 1999.

Polakova, K., Plaksin, D., Chung, D.H., Belyakov, I., Berzofsky, J.A., and Margulies, D.H. Antibodies directed against H-2D^{d} complexed with an immunodominant peptide from human immunodeficiency virus envelope glycoprotein 120: Similarities to a T-cell receptor with the same specificity. *J. Immunol.* 165: 5703-5712, 2000.

Natarajan, K., Sawicki, M., Margulies, D. H. and Mariuzza, R. A. Crystal structure of human CD69: A C-type lectin-like activation marker of hematopoietic cells. *Biochemistry* 39: 14779-14786, 2000.

McHugh, R.S., Shevach, E.M., Margulies, D. H., and Natarajan, K. A TCR transgenic mouse model for organic specific autoimmunity. *Eur. J. Immunol.*, 31: 2094-2103, 2001.

Ethan M. Shevach, M.D.

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Dr. Shevach received his M.D. degree from Boston University in 1967. Following clinical training, he joined the Laboratory of Immunology as a Senior Staff Fellow in 1972, was appointed a Senior Investigator in 1973, and became a Section Head in 1987. Dr. Shevach served as Editor-in-Chief of the *Journal of Immunology* from 1987 to 1992 and is presently Editor-in-Chief of *Cellular Immunology*.

Description of Research Program

The development of autoimmune disease involves a breakdown in the mechanisms that control self versus nonself discrimination. One of the major mechanisms for the control of autoreactivity and the prevention of autoimmune disease is the existence of suppressor/regulatory T cells that suppress autoreactive T cells that have escaped passive mechanisms of tolerance induction. The major goal of this Section is to understand the mechanism of action of these regulatory T cells in preventing organ-specific autoimmunity. We have identified a unique subpopulation of CD4+ T cells that co-express CD25 and are capable of suppressing the induction of autoimmune disease *in vivo.* Model *in vitro* systems have been developed that mimic the function of these cells *in vivo.* CD4+CD25+ T cells suppress polyclonal and antigen-specific responses by a novel cytokine-independent, cell contact-dependent mechanism. The antigen specificity and the precise mechanisms of suppression utilized by the CD4+CD25+ cells are the subjects of ongoing investigations. While regulatory T-cells play a major role in preventing autoimmune disease, cytokines are the major mediators of organ-specific damage. A second focus of the Section's studies is the role of cytokine networks, bridging innate and acquired immune systems, in the pathogenesis of organ-specific autoimmunity, particularly experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. We have focused on the role of IL-12 and IL-18 and their cellular receptors in the development of T helper 1 cells that mediate EAE. Our goals for the future include the development of therapeutic protocols for the manipulation of cytokine/chemokine networks resulting in inhibition of disease induction and modulation of established disease.

Major Areas of Research

- Pathogenesis and treatment of autoimmune diseases
- Suppressor/regulatory T-cell function
- Cytokine networks in autoimmunity

Selected Recent Publications

Smeltz, R.B., Chen, J., Hu-Li, J., Shevach, E.M. Regulation of interleukin (IL)-18 receptor alpha chain expression on CD4 (+) T cells during T helper (Th)1/Th2 differentiation. Critical downregulatory role of IL-4. *J. Exp. Med.* 194: 143-53, 2001.

Piccirillo, C.A., Shevach, E.M. Cutting edge: control of cd8(+) T cell activation by cd4(+)cd25(+) immunoregulatory cells. *J. Immunol.* 167: 1137-40, 2001.

Shevach, E.M. Regulatory T cells in autoimmunity. *Ann. Rev. Immunol.* 18:423-449, 2000.

Chang, J.T., Shevach, E.M., and Segal, B.M. Regulation of interleukin-12 receptor b2 subunit expression by endogenous IL-12: A critical step in the differentiation of pathogenic autoreactive T cells. *J. Exp. Med.* 189: 969-978, 1999.

Thornton, A.M. and Shevach, E.M. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting IL-2 production. *J. Exp. Med.* 188: 287-296, 1998.

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Dr. Sitkovsky obtained his Ph.D. from the Moscow State University in the Soviet Union in 1972 for the study of the biochemistry and cell physiology during oxidative stress. Following postdoctoral research at Moscow State University and MIT, he joined the LI as a Senior Investigator and became a Section Head in 1991.

Description of Research Program

Molecular Mechanisms of Cytotoxic T Cell Generation and Effector Functions. Our focus is on understanding the mechanisms by which CTLs or T-killer cells are generated and recognize and kill cancerous and virus-infected target cells but spare innocent bystanders. We are identifying enzymes and cell surface receptors that play important functional roles in CTL generation and functions.

Response to Nonimmune Signals as a Mechanism of Lymphocyte Adaptation to Different Local Tissue Extracellular Environments. The main hypothesis stems from the realization that lymphocytes are exposed not only to antigen and cytokines but also to many other signals from the local tissue extracellular environment. Accordingly, it is important to understand the regulatory role of physiologically abundant signaling molecules in local tissue extracellular environments that are designated as nonimmune.

Phosphorylation of the Ectodomains of Functionally Important Surface Proteins. Extracellular ATP is also considered in its novel role as a phosphate donor in phosphorylation of ectodomains of functionally important cell surface proteins. T-cell differentiation and effector functions involve multiple cell-cell contacts and interactions mediated by cell surface-located recognition structures, cell adhesion molecules, and growth factor receptors. It is hypothesized that phosphorylation of the extracellular domains (ectodomains) of these surface proteins may be involved in regulation of T-cell cognate interactions and effector functions, and evidence has been provided of constitutive serine and threonine phosphorylation of the T-cell antigen receptor (TCR) ectodomains. TCR ectodomain phosphorylation may be a mechanism of regulation for TCR-mediated immune responses. Testing of this model is in progress in studies of functional responses of transfectants, which express TCR with mutated ectophosphorylation sites.

Physiologically Low Oxygen Tensions in Lymphoid Organs In Vivo. Because adenosine accumulation is associated with hypoxic conditions, Section scientists performed direct measurements of oxygen tension in lymphoid tissues. Surprisingly, hypoxic conditions were found in all areas of thymus, spleen, and lymph nodes, raising questions about the suitability of routinely used *in vitro* studies under "normoxic" conditions (about 21 percent oxygen in CO2 incubators) in recreating *in vivo* conditions. These findings also point to the need to explore the mechanism of adaptation of lymphocytes and of regulation of immune response by oxygen sensors in lymphoid cells.

Major Area of Research

- Mechanism of T-cell differentiation into antigen-specific effector T cells with emphasis on the role of "nonimmune" factors (oxygen tension, purinergic receptors) in local tissues extracellular environment

Selected Recent Publications

Apasov, S.G., Blackburn, M.R., Kellems, R.E., Smith, P.T., Sitkovsky, M.V. Adenosine deaminase deficiency increases thymic apoptosis and causes defective T-cell receptor signaling. *J. Clin. Invest.* 108:131-41, 2001.

Armstrong, J.M., Chen, J.F., Schwarzschild, M.A., Apasov, S., Smith, P.T., Caldwell, C., Chen, P., Figler, H., Sullivan, G., Fink, S., Linden, J., Sitkovsky, M. Gene dose effect reveals no Gs-coupled A2A adenosine receptor reserve in murine T-lymphocytes: studies of cells from A2A-receptor-gene-deficient mice. *Biochem. J.* 354 (Pt 1): 123-30, 2001.

Laboratory of Immunopathology

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Janet W. Hartley, Ph.D., Emerita

Molecular Pathology Section
Victor V. Lobanenkov, Ph.D., Head

Research Activities

The Laboratory of Immunopathology (LIP) conducts basic research on mechanisms governing normal cell growth and differentiation and their deregulation during neoplastic transformation. A major emphasis of the Virology and Cellular Immunology Section, headed by Dr. Morse, is on hematopoietic neoplasms in the mouse, particularly those of B-cell origin. Sources of lymphomas include spontaneous neoplasms of inbred and congenic mice, transgenics expressing genes involved in human neoplasia and gene knockouts. Approaches to understanding pathogenesis encompass histologic, phenotypic and molecular genetic analyses including spectral karyotyping and cDNA microarray. Recent studies have led to the understanding of human-mouse parallels for B- and T-cell lymphomas. Additional work focuses on the roles of retroviruses in lymphomagenesis and immunodeficiency in mice. Studies in the Molecular Pathology Section, headed by Dr. Lobanenkov, relate to the cellular, developmental and molecular biology of the transcription factor, CTCF. CTCF functions in transcriptional activation and repression to regulate cell growth. It also acts as a universal insulator and reads imprinted sites. Recent work has identified CTCF as a candidate tumor suppressor gene involved in several different types of cancer. Current work is directed at understanding how CTCF performs its many functions by identifying and analyzing target sites, evaluating the contributions of DNA methylation to specification of function and determining protein partners. Additional work in cancer is directed at understanding mutations in CTCF target sites and CTCF itself. A wide range of cell biologic and molecular approaches is utilized to develop these studies including generation of gene-targeted mice.

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Dr. Morse graduated from Harvard Medical School and then completed his internship and residency at Peter Bent Brigham Hospital. Following postdoctoral studies in the NIAID, he joined the NIAID Laboratory of Viral Diseases in 1980 and became Chief of the LIP in 1985.

Description of Research Program

This program focuses on alterations in the development and function of the immune system caused by infections– particularly by retroviruses, autoimmunity, or mutations. Of particular interest are studies aimed at elucidating the basic cellular mechanisms involved in the development of lymphoproliferation and immunodeficiency. Research in this area involves identifying target cells for virus infections, defining the role of host genes in regulation of virus expression, evaluating the role of cytokines as determinants of disease, and examining signaling pathways involved in cell activation, growth, and differentiation.

Major accomplishments include identification of a defective virus as the cause of an immunodeficiency disease of mice, mouse AIDS (MAIDS); demonstration that IFN-γ is a major determinant of susceptibility and resistance to MAIDS; elucidation of critical determinants of CD4+ T cell-B cell interaction in MAIDS; and characterization of IL-12 as an immunomodulator for prevention and treatment of retrovirus-induced disease.

Future directions are to elucidate the cellular and molecular mechanisms involved in the immunopathogenesis of retrovirus-induced disease; characterize host genes identified in yeast two-hybrid systems that bind to retroviral Gag proteins; and evaluate cell signaling pathways altered by retrovirus infections of lymphocytes and macrophages. Analyses of IFN signaling pathways in mice with a null mutation in IRF family members and in humans with chronic myelogenous leukemia are of increasing importance.

Major Areas of Research

- Mouse models of retroviral pathogenesis
- Normal hematopoietic differentiation
- Mechanisms of lymphoma/leukemia development in mice and humans

Selected Recent Publications

Morse, H.C., Qi, C., Chattopadhyay, S.K., Hori, M., Taddesse-Heath, L., Ozato, K., Hartley, J.W., Taylor, B.A., Ward, J.M., Jenkins, N.A., Copeland, N.G., Fredrickson, T.N. Combined histiologic and molecular features reveal previously unappreciated subsets of lymphoma in AKXD recombinant inbred mice. *Leuk. Res.* 25:719-33, 2001.

Hori, M., Xiang, S., Qi, C.F., Chattopadhyay, S.K., Fredrickson, T.N., Hartley, J.W., Kovalchuk, A.L., Bornkamm, G.W., Janz, S., Copeland, N.G., Jenkins, N.A., Ward, J.M., Morse, H.C. 3rd. Non-Hodgkin lymphomas of mice. *Blood Cells Mol. Dis.* 27: 217-22, 2001.

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Klenova, E.M., Chernukhin, I.V., El-Kady, A., Lee, R.E., Pugacheva, E.M., Loukinov, D.I., Goodwin, G.H., Delgado, D., Filippova, G.N., Leon, J., Morse, H.C. 3rd, Neiman, P.E., Lobanenkov, V.V. Functional phosphorylation sites in the C-terminal region of the multivalent multifunctional transcriptional factor CTCF. *Mol. Cell. Biol.* 21:2221-34, 2001.

Kovalchuk, A.L., Qi, C.F., Torrey, T.A., Taddesse-Heath, L., Feigenbaum, L., Park, S.S., Gerbitz, A., Klobeck, G., Hoertnagel, K., Polack, A., Bornkamm, G.W., Janz, S., Morse, H.C. 3rd. Burkitt lymphoma in the mouse. *J. Exp. Med.* 192:1183-90, 2000.

Victor Lobanenkov, Ph.D.

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Dr. Lobanenkov received a master's degree in nuclear physics from the Institute of Physics and Engineering in 1977 and a Ph.D. in experimental oncology from the Cancer Research Center in Moscow in 1981. He was Head of the Molecular Carcinogenesis group at the Institute of Carcinogenesis: All-Union Cancer Center of the former USSR and a Visiting Scholar at the Royal Cancer Hospital of London until 1990. He then was invited to work at the Fred Hutchinson Cancer Research Center in Seattle, WA, where he was Foreign Faculty in Residence funded by NIH grants. In 1999, he became Head of the Molecular Pathology Section in the LIP.

Description of Research Program

Dr. Lobanenkov has focused the work of his Section on structure and function of the *CTCF* gene that he identified in 1990 and initially characterized as negative transcriptional regulator of the *MYC* oncogene. CTCF is a uniquely versatile transcriptional regulator with diverse functions linked to epigenetics and disease. It is an exceptionally evolutionarily conserved zinc finger (ZF) phosphoprotein that binds *via* combinatorial utilization of the eleven ZFs to ~ 50 bp long DNA target sites with remarkable sequence variation. Formation of different CTCF-DNA complexes, a subset of which is CpG-methylation-sensitive, results in distinct functions including gene activation, repression, silencing or chromatin insulation. Disrupting the spectrum of target specificities by ZF mutations or by abnormal selective methylation of certain CTCF-targets is associated with cancer. CTCF emerged, therefore, as a central player in networks linking expression domains with epigenetics and cell growth regulatory processes. It is thus not surprising that in recent years there has been a rapidly growing interest in the *CTCF* gene. In additional to LIP, different aspects of CTCF biology have become a major focus of research in several other laboratories both within and outside NIH and abroad.

In 1999, Dr. Lobanenkov established a program directed to better understanding of CTCF normal function in development, cell-cycle regulation, and gene imprinting; and of CTCF malfunction in cancer, and in other human diseases associated with abnormal site-specific DNA methylation such as congenital myotonic dystrophy. This program takes advantage of cancer-prone mouse *CTCF* knock-out models, and of *Drosophila* genetics based on identification and cloning of the *CTCF* homologue in flies. Identification and functional characterization of cancer-associated CTCF mutations can be viewed as experiments of nature that reveal critically important CTCF target genes and protein partners that are involved in regulation of CTCF function. Identifying such genes and partners reveals regulatory gene networks and pathways which define tumor phenotype(s), and thus leads the Section well beyond studies of CTCF *per se* because novel genes in a pathway of mutant CTCF are to be, in turn, potential oncogenes or tumor suppressor genes.

Major Areas of Research

- Role of CTCF in cancer and DNA-methylation-dependent diseases
- Mechanisms of transcriptional regulation by CTCF and of *CTCF*

Selected Recent Publications

Klenova, E.M., Chernukhin, I.V., El-Kady, A., Lee, R.E., Pugacheva, E.M., Loukinov, D.I., Goodwin, G.H., Delgado, D., Filippova, G.N., Leon, J., Morse, H.C. 3rd, Neiman, P.E., Lobanenkov, V.V. Functional phosphorylation sites in the C-terminal region of the multivalent multifunctional transcriptional factor CTCF. *Mol. Cell. Biol.* 21: 2221-2234, 2001.

Ohlsson, R., Renkawitz, R., and Lobanenkov, V. CTCF is a uniquely versatile transcription regulator linked to epigenetics and disease. *Trends in Genetics* 17: 520-527, 2001.

Filippova, G.N., Thienes, C.P., Penn, B.H., Cho, D.H., Hu, Y.J., Moore, J.M., Klesert, T.R., Lobanenkov, V.V., Tapscott, S.J. CTCF-binding sites flank CTG/CAG repeats and form a methylation-sensitive insulator at the *DM1* locus. *Nature Genet*. 28:335-43, 2001.

Laboratory of Immunoregulation

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Immune Activation Section
Ulrich K. Siebenlist, Ph.D., Head

Immunologic Diseases Section
Michael C. Sneller, M.D., Head

Immunopathogenesis Section
Anthony S. Fauci, M.D., Head

International HIV and STD Unit
Thomas C. Quinn, M.D., Head

Research Activities

The major theme of the Laboratory of Immunoregulation (LIR) continues to be the elucidation of cellular and molecular mechanisms of the regulation of the human immune response in health and disease. A major component of these efforts is focused on the study of immunopathogenic mechanisms of human immunodeficiency virus (HIV) infection and disease progression. The scientific basis for the rational design of strategies aimed at the prevention and treatment of HIV infection depends on delineating these fundamental events whereby HIV destroys the human immune system. Our investigation of host factors involved in the evolution of HIV disease indicates that HIV pathogenesis is a multifactorial and multiphasic process. Particularly important aspects of this process that are under intense investigation include the role of endogenous cytokines and chemokines in regulating HIV replication; the regulation of the expression of HIV coreceptors; the elucidation of HIV envelope-mediated intracellular signaling events that are responsible for immune dysfunction; the role of a latent, inducible reservoir of HIV-infected cells in the pathogenesis of HIV disease and its implications for antiretroviral therapy; the role of HIV infection of various cells of the immune system in disease pathogenesis; and the role of immunomodulation in immune reconstitution during antiretroviral therapy for HIV infection. LIR also conducts clinical trials to determine the safety and efficacy of drugs for the treatment of HIV infection and its complications and develops methods for immunologic reconstitution in HIV-infected individuals. International studies of the epidemiology and pathogenesis of HIV infection and other sexually transmitted diseases are an additional area of focus. Studies on the fundamental nature of normal B-cell and T-cell activation continue to be important ongoing components of the LIR research agenda. Progress continues to be made in understanding the role of dysregulated immunity in the vasculitic syndromes; this progress has made possible the design and execution of rational therapeutic strategies for these disease states.

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Dr. Fauci received his A.B. from College of the Holy Cross and his M.D. degree from Cornell University Medical College. He then completed an internship and residency at The New York Hospital-Cornell Medical Center. In 1968, Dr. Fauci came to the NIH as a clinical associate in the LCI at NIAID. In 1974, he became Head of the Clinical Physiology Section, LCI, and in 1977, he was appointed Deputy Clinical Director of NIAID. In 1980, he was appointed Chief of the Laboratory of Immunoregulation, a position he still holds. Dr. Fauci became Director of NIAID in 1984.

Description of Research Program

The Section's research involves the delineation of the mechanisms of activation, proliferation, and differentiation of human lymphoid cells in the normal immune response as well as in diseases characterized by abnormalities of immune function. These studies range from basic cellular physiology up through and including the molecular biology of lymphoid cell function.

Specific areas of focus include the immunopathogenic mechanisms of human immunodeficiency virus infection, together with studies of the clinical, virologic and therapeutic aspects of the acquired immunodeficiency syndrome; the mechanisms of immunosuppression by various agents, such as corticosteroids and cytotoxic drugs and correlation of the suppression of populations of lymphoid cells with disease activity and with alterations in host defense mechanisms; and the pathogenesis and specific therapeutic approaches to hypersensitivity vasculitic and granulomatous diseases, such as Wegener's granulomatosis and the polyarteritis nodosa group of inflammatory vasculitides.

Major Areas of Research

- HIV immunopathogenesis
- Mechanisms involved in normal and abnormal immune responses
- Pathogenesis of vasculitic and granulomatous diseases

Selected Recent Publications

Moir, S., Malaspina, A., Ogwaro, K.M., Donoghue, E.T., Hallahan, C.W., Ehler, L.A., Liu, S., Adelsberger, J., Lapointe, R., Hwu, P., Baseler, M., Orenstein, J.M., Chun, T.-W., Mican, J.A., Fauci, A.S. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. *Proc. Natl. Acad. Sci. USA* 98: 10362-7, 2001.

Chun, T.-W., S. J. Justement, S. Moir, C.W. Hallahan, L.A. Ehler, S. Liu, M. McLaughlin, M. Dybul, J.M. Mican, and A. S. Fauci. Suppression of HIV replication in the resting CD4+ T cell reservoir by autologous CD8+ T cells: implications for the development of therapeutic strategies. *Proc. Natl. Acad. Sci. USA* 98: 253-258, 2001.

Fauci, A.S. Infectious diseases: considerations for the 21st century. *Clin. Infect. Dis.* 32:675-85, 2001.

Chun, T.-W., R.T. Davey Jr., M. Ostrowski, J.S. Justement, D. Engel, J.I. Mullins, and A.S. Fauci. Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy. *Nature Med.* 6:757-761, 2000.

Moir, S., A. Malaspina, Y. Li, T.-W. Chun, T. Lowe, J. Adelsberger, M. Baseler, L.A. Ehler, S. Liu, R.T. Davey Jr., J.A. Mican, and A.S. Fauci. B cells of HIV-1-infected patients bind virions through CD21-complement interactions and transmit infectious virus to activated T cells. *J. Exp. Med.* 192: 637-646, 2000.

Mark Connors, M.D.

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Dr. Connors received his M.D. from Temple University and was trained in pediatrics at Tuft's New England Medical Center. He joined the Laboratory of Infectious Diseases in 1989 to study the immune response to respiratory syncytial virus. He was trained in infectious diseases at the NIH Clinical Center and at the Children's Hospital of Philadelphia. He joined the LIR in 1995 to study the human immune response to HIV.

Description of Research Program

Identification of the components, targets, and magnitude of an effective immune response to HIV are important steps toward the development of an effective vaccine. Although patients with normal $CD4^+$ T-cell counts and low levels of plasma virus are a heterogeneous group, a small subgroup of patients with truly non-progressive HIV infection and restriction of virus replication likely hold important clues to the basis of an effective immune response to HIV. For this reason, we have focused considerable attention on groups of such patients who are felt to have immune system-mediated restriction of virus replication. It now appears clear that a large fraction of patients previously considered long-term non-progressors (LTNPs) ultimately show a decline of CD4+ T-cell numbers. A small subpopulation (fewer than 0.8% of HIV-infected individuals) shows no signs of progression over a 10-year period. We have assembled a unique cohort of such patients with non-progressive disease. Many of these patients have been infected for 15 years with no CD4+ T-cell decline, plasma virus load below the level of detection, and no virus cultured in routine CD8+ T-cell depleted cultures. We have previously shown that these patients are unique in the ability of their CD8+ T cells to restrict HIV replication when engrafted into experimental animals. Cells from these patients, all of whom are untreated, are being used to systematically dissect the mechanisms of immune-mediated restriction of virus replication.

Major Areas of Research

- T-cell mediated mechanisms of resistance in patients with effective immunity to HIV and nonprogressive disease.

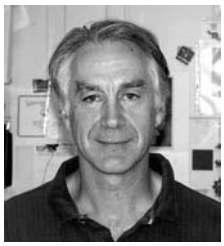
Selected Recent Publications

Lopez, J.C., Shupert, W.L., McNeil, A.C., Flanigan, M., Savage, A., Martino, L., Weiskopf, E.E., Adelsberger, J., Stevens, R., Gea-Banacloche, J.C., Davey, R. Jr., and Connors, M. Resistance to replication of HIV challenge virus in SCID-Hu mice engrafted with PBMC of nonprogressors is mediated by $CD8^+$ T cells and associated with a proliferative response to p24 antigen. *J. Virol.* 74: 2023-2028, 2000.

Migueles, S.A., Sabbaghian, M.S., Shupert, W.L., Bettinotti, M.P., Marincola, F.M., Schwartz, D., Sullivan, J., and Connors, M. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV infected long-term nonprogressors. *Proc. Natl. Acad. Sci. USA* 97:2709-2714, 2000.

Gea-Banacloche, J.C., Martino, L., Migueles, S., Shupert, W.L., Prussin, C., Stevens, R., Lambert, L., Bernaldo de Quiros, J.C.L., and Connors, M. Maintenance of large numbers of virus specific $CD8^+$ T-cells in HIV-infected progressors and long-term nonprogressors. *J. Immunol.* 165: 1082-1092, 2000.

Gea-Banacloche, J.C., Martino, L., Mican, J.M., Hallahan, C.W., Baseler, M., Stevens, R., Lambert, L., Lane, H.C., and Connors, M. Longitudinal changes in $CD4^+$ T cell antigen receptor diversity and naïve/memory cell phenotype following 24 months of antiretroviral therapy of HIV-infected patients. *AIDS Res.* 16: 1877-1886, 2000.



John H. Kehrl, M.D.

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Dr. Kehrl received his M.D. from Wayne State University and completed training in internal medicine at Yale New Haven Hospital. He joined the Laboratory of Clinical Investigation as a Clinical Associate in 1980 and the Laboratory of Immunoregulation in 1981. In 1991, Dr. Kehrl was tenured in NIAID and made a Section Chief in the Laboratory of Immunoregulation in 1994.

Memberships: Alpha Omega Alpha, American Federation for Clinical Research, American Society for Clinical Research, American Association of Immunologists,

Research Group Members: Chong-Shan Shi, Hyeseon Cho, Chantal Moratz, Srikumar Sinnarajah, Deepa Srikumar, Shi Geng-Xian, Kathleen Harrison, Gaye Lynn Wilson

Description of Research Program

Our goals are to understand how G-protein coupled receptors (GPCR) transduce signals to downstream effectors such as the MAP kinases, to discern the mechanisms that control these responses, to understand how G-protein activation leads to transcriptional responses, and to determine the physiologic consequences of G-protein activation for lymphocyte function. Members of the Section identified a family of proteins that regulate heterotrimeric G-protein signaling, which have been termed RGS proteins. They are GTPase activating proteins (GAPs) for Gα subunits of heterotrimeric G proteins. By shortening the duration that a Gα subunit is GTP bound the RGS proteins curtail both Gα and Gβγ signaling. RGS proteins also affect heterotrimeric G-protein signaling via other mechanisms. They may act as effector antagonists as well as directly interact with downstream effectors such as adenylyl cyclases. In addition, certain RGS proteins directly regulate Gβγ signaling. There are approximately 20 RGS family members and 7 members are abundantly present in lymphocytes, where they regulate responses to chemokines.

Other major accomplishments of the BCMIS include the identification of the initial member of a subfamily of protein kinases termed germinal center kinases (GCK), the identification of a second member of this subfamily termed GCKR, a demonstration that GCK and GCKR participate in signaling through the tumor necrosis factor and CD40 receptors to activate Jun kinase, development of BSAP transgenic mice, the isolation of the transcription factor HlxB9 (HB9), and development of HlxB9 deficient mice, which revealed a requirement for it in pancreas development, β-cell function, and motor neuron development.

Major Areas of Research

- G-protein signaling and the role of RGS proteins
- Jun kinase activation by GCK kinases
- Transcriptional control in lymphocytes

Selected Recent Publications

Druey, K.M., Blumer, K.J., Kang, V.H., and Kehrl, J.H. Inhibition of G-protein-mediated MAP kinase activation by members of a novel mammalian gene family. *Nature* 379: 742-746, 1996.

Kehrl, J.H. Heterotrimeric G-protein signaling: roles in immune function and fine-tuning by RGS proteins. *Immunity* 8: 1-10, 1998.

Harrison, K.A., Thaler, J., Pfaff, S.L., Gu, H., Kehrl, J.H. Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in Hlxb9-deficient mice. *Nature Genetics* 23:71-5, 1999.

Sinnarajah, S., Dessauer, C.W., Srikumar, D., Chen, J., Yuen, J., Yilma, S., Dennis, J.C., Morrison, E.E., Vodyanoy, V., and Kehrl, J.H. RGS2 regulates signal transduction in olfactory neurons by attenuating adenylyl cyclase III activation. *Nature* 49: 1051-1055, 2001.

H. Clifford Lane, M.D.

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Dr. Lane received his M.D. degree from the University of Michigan in 1976. He then completed an internship and residency at the University of Michigan Hospital, Ann Arbor, Michigan. In 1979, Dr. Lane came to the NIH as a clinical associate in the LIR, NIAID. In 1985, he was appointed Deputy Clinical Director, NIAID and in 1989, he became the Head of the Clinical and Molecular Retrovirology Section (CMRS) of the LIR, a position he still holds. In 1991, Dr. Lane became Clinical Director of NIAID and in 1998 was promoted to the rank of Assistant Surgeon General in the United States Public Health Service. He is currently on the editorial boards of the *Journal of Acquired Immune Deficiency Syndromes and Clinical Immunology and Immunopathology.*

Description of Research Program

In the laboratory, Dr. Lane's early work involved studies aimed at dissecting the normal immunoregulatory mechanisms controlling the human immune response to specific antigen challenge. Within a brief time, the AIDS epidemic emerged and Dr. Lane became one of the early investigators to study immunopathogenic mechanisms of HIV disease. At present his studies focus on the mechanisms underlying immune reconstitution in patients with HIV infection and the effects of IL-2 on the immune system.

In the clinical arena, Dr. Lane explores innovative approaches to therapy and has used experimental therapeutic interventions as a means of furthering our understanding of HIV pathogenesis. These include the strategies of syngeneic bone marrow transplantation and the adoptive transfer of lymphocytes, and the role of the cytokines alpha interferon and IL-2 in the treatment of patients with HIV infection.

Major Areas of Research

- Pathogenesis of HIV infection emphasizing mechanisms of immunodeficiency
- Immunologic approaches to therapy for HIV infection

Selected Recent Publications

Lempicki, R.A., J.A. Kovacs, M.W. Baseler, J.W. Aldesberger, R.L. Dewar, V. Natarajan, M.C. Bosche, M.A. Metcalf, R.A. Stevens, L.A. Lambert, W.G. Alvord, M.A. Polis, R.T. Davey, Jr., D.S. Dimitrov and H.C. Lane. Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of CD4+ and CD8+ T cell turnover in HIV-infected patients. *Proc. Natl. Acad. Sci. USA* 97: 13778-13783, 2000.

Sereti, I., J.C. Gea-Banacloche, M-Y. Kan, C.W. Hallahan and H.C. Lane. Interleukin-2 leads to dose dependent expression of the alpha chain of the IL-2 receptor on CD25 negative T lymphocytes in the absence of antigenic stimulation. *Clin. Immunol.* 97:266-276, 2000.

Imamichi, H., K.A. Crandall, V. Natarajan, M.K. Jiang, R.L. Dewar, S. Berg, A. Gaddam, M. Bosche, J.A. Metcalf, R.T. Davey, Jr. and H.C. Lane. The viral quasispecies of HIV-1 rebounding following discontinuation of HAART are similar to the viral quasispecies present prior to the initiation of therapy. *J. Infect. Dis.* 183: 36-50, 2001.

Imamichi T., M. Murphy, H. Imamichi and H. C. Lane. Amino acid deletion at codon 67 and thr-to-gly change at codon 69 of HIV-1 reverse transcriptase confer novel drug resistance profile. *J. Virol.* 75: 3988-3992, 2001.

Thomas C. Quinn, M.D., M.Sc.

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Dr. Quinn obtained his M.D. from Northwestern University. He was a Research Associate in infectious diseases from 1977 to 1979 in NIAID's Laboratory of Parasitic Diseases and completed a fellowship in infectious diseases at the University of Washington in Seattle from 1979 to 1981. He is currently Senior Investigator and Head of the International HIV and STD Unit in the LIR. Since 1981, Dr. Quinn has been assigned to the Division of Infectious Diseases at The Johns Hopkins University School of Medicine, where he became Professor of Medicine in 1991. His awards include: AOA Epidemiology Research Award, Charles C. Shepard Science Award, James H. Nakano Citation for Outstanding Scientific Publication; USPHS Outstanding Service Award, USPHS Meritorious Service Award; USPHS Distinguished Service Award.

Description of Research Program

Internationally, there are 36 million HIV-infected individuals and 24 million people have already died from AIDS. A major focus within our laboratory has been to define the unique epidemiologic, clinical, virologic, and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In a multicenter study on perinatal transmission in the U.S., we determined that elevated RNA viral levels at birth were suggestive of in utero infection and that a high plasma RNA viral load in the first two months of life (> 300,000 copies per ml) was strongly associated with rapid progression to AIDS or death.

In a community-based trial of mass STD treatment in the Rakai district of Uganda, we documented relatively high rates of chlamydia and gonorrhea in 15-24 year-olds (5%). Future studies will address the effectiveness of mass treatment in limiting HIV transmission.

We identified a gender difference in HIV viral load between men and women in a cross-sectional study and the predictive value of an RNA level for progression to AIDS appeared to be different in men and women. We then conducted a nested case-controlled study of RNA levels in both male and female injecting drug users to provide a longitudinal perspective on viral levels in men and women who progress to AIDS compared to male and female non-progressors. We found that plasma viral load at seroconversion in women is not as predictive of progression to AIDS as it is in men and consequently viral load differences in women compared to men need to be taken into account when considering when to initiate antiretroviral therapy.

We recently demonstrated a direct correlation between *C. pneumoniae* and atherosclerotic heart disease by the isolation of *C. pneumoniae* in an explanted heart with severe coronary atherosclerosis. Future studies will continue to examine the etiologic role and immunopathogenesis of *C. pneumoniae* in atherosclerosis.

Major Areas of Research

- International and U.S. epidemiologic studies of HIV and STDs
- Chlamydia epidemiology and pathogenesis

Selected Recent Publications

Quinn, T.C., Brookmeyer, R., Kline, R., Shepherd, M., Paranjape, R., Mehendale, S., Gadkari, D.A., Bollinger, R.C. Feasibility, accuracy and cost savings of pooling sera for HIV-1 viral RNA for the diagnosis of acute primary HIV-1 infection and estimation of HIV-1 incidence. *AIDS* 14: 2751-2757, 2000.

Sterling, T.R., Vlahov, D., Astemborski, J., Hoover, D.R., Margolick, J.B., Quinn, T.C. The relationship between initial HIV-1 RNA level after seroconversion and progression to AIDS in women and men: implications for initiation of antiretroviral therapy. *N. Engl. J. Med.* 344:720-5, 2001.

Burnett, M.S., Gaydos, C.A., Madico, G.E., Paigen, B., Quinn, T.C., Epstein, S.E. Atherosclerosis in ApoE knockout mice infected with multiple pathogens. *J. Infect. Dis.* 183:226-231, 2001.

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Dr. Siebenlist received his Ph.D. at Harvard University, studying protein-DNA interactions with the Nobel Laureate Dr. Walter Gilbert. As a postdoctoral fellow in Dr. Philip Leder's laboratory both at NIH and Harvard Medical School, Dr. Siebenlist studied immunoglobulin gene structures and the regulation of the *myc* oncogene. He then joined the LIG where he is currently the Head of the Immune Activation Section.

Description of Research Program

This laboratory focuses on the elucidation of molecular mechanisms that operate during the development of the immune system and during immune activation in health and disease. In particular, we try to understand the developmental signals and programs required for the generation of immune-competent cells (such as T, B lymphocytes), and we try to understand the responses of competent cells to various pathogens. Investigations are centered on (1) identification of signal transduction pathways initiated during immune responses or in development leading to activation of transcription factors; (2) functional characterization of the genetic programs induced by the activated transcription factors. One family of transcription factors of particular interest is NF-κB, since these factors have many critical functions in the development and maintenance of the immune system, as well as in innate and adaptive immune responses. As demonstrated in this laboratory, for example, NF-κB factors are required for the development of competent B cells and osteoclasts and for the microarchitectures of secondary lymphoid organs. In addition, these factors are critical in cellular stress responses and they are co-opted by viruses such as HIV and HTLV. Specific functions of NF-κB under study in these various biologic contexts include control of apoptosis, proliferation, differentiation and host defense. Our research on these factors provides a window on an entire coordinated immune activation program. To probe the *in vivo* biologic roles of these factors and their signaling pathways, we study immune challenged mice deficient in NF-κB factors or in component parts of their signaling pathways. Ultimately, we seek to understand the critical molecular targets of NF-κB in response to a specific immune challenge. In addition, we seek to elucidate the pathways by which various distinct signals activate transcription factors such as NF-κB. These approaches not only help to understand development and roles of immune-relevant cells in health, they also lay the foundation to gain critical insights into immune deficiencies or inflammatory diseases, especially since NF-κB factors are often inappropriately regulated in such situations. These studies also identify possible specific targets for therapeutic intervention aimed to control NF-κB activity. As some of the genes encoding the NF-κB factors or their regulators have been associated with recurrent genetic alterations in certain tumors, our studies also provide clues for how tumors are formed.

Major Areas of Research

- Development of immune cells
- Signal transduction
- NF-κB transcription factors

Selected Recent Publications

Ellinger-Ziegelbauer, H., Kelly, K., Siebenlist, U. Cell cycle arrest and reversion of Ras-induced transformation by a conditionally activated form of mitogen-activated protein kinase kinase kinase 3. *Mol. Cell. Biol.* 19:3857-68, 1999.

Poljak, L., Carlson, L., Cunningham, K., Kosco-Vilbois, M.H., Siebenlist, U. Distinct activities of p52/NF-kappa B required for proper secondary lymphoid organ microarchitecture: functions enhanced by Bcl-3. *J. Immunol.* 63:6581-6588, 1999.

Leonardi, A., Chariot, A., Claudio, E., Cunningham, K., Siebenlist, U. CIKS, a connection to Ikappa B kinase and stress-activated protein kinase. *Proc. Natl. Acad. Sci. USA* 97:10494-10499, 2000.

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Dr. Sneller obtained his undergraduate and medical education at the University of Kansas. He completed his postgraduate training in internal medicine and rheumatology at Duke University and received further training in infectious diseases at the NIAID.

Description of Research Program

The systemic vasculitides include a variety of generalized, life-threatening autoimmune diseases that have been studied for more than 30 years at the NIAID. LIR investigators have previously demonstrated that the use of daily cyclophosphamide and glucocorticoids is highly effective therapy for Wegener's granulomatosis (WG), a disease that previously was nearly uniformly fatal. These studies pioneered the use of low-dose cytotoxic therapy as a treatment for life-threatening autoimmune diseases; this treatment paradigm is now accepted as an international gold standard. A major focus within the Immunologic Diseases Section has been to develop less toxic therapeutic regimens for the treatment of WG and related systemic vasculitis syndromes. Long-term follow-up of patients treated with our low-dose weekly methotrexate regimen indicates that this regimen is effective as initial therapy for selected patients with WG and represents a less toxic alternative to standard cyclophosphamide therapy in this group. Additional studies performed by the Immunologic Diseases Section have demonstrated that methotrexate is also effective as maintenance therapy in patients with severe disease in whom cyclophosphamide has induced disease remission. The Section has recently initiated several new studies including a phase II trial to assess the comparative efficacy of methotrexate versus mycophenolate mofetil for maintaining remission in patients with WG and related vasculitides and a phase I/II study of the efficacy of etanercept and methotrexate in the treatment of WG.

Major Areas of Research

- Pathogenesis and treatment of systemic vasculitis syndromes

Selected Recent Publications

Sneller, M.C., Hoffman, G.S., Talar-Williams, C., Kerr, G.S., Hallahan, C.W., and Fauci, A.S. Analysis of 42 Wegener's granulomatosis patients treated with methotrexate and prednisone. *Arthritis Rheum.* 38: 601-613, 1995.

Talar-Williams, C., Hijazi, Y.M., Walther, M.M., Linehan, W.M., Hallahan, C.W., Lubensky, I., Kerr, G.S., Hoffman, G.S., Fauci, A.S., and Sneller, M.C. Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener's granulomatosis. *Ann. Intern. Med.* 124: 477-484, 1996.

Ludviksson, B.J., Sneller, M.C., Chua, K.S., Talar-Williams, C., Langford, C.A., Ehrhardt, R.O., Fauci, A.S., and Strober, W. Active Wegener's granulomatosis is associated with HLA-DR$^+$ CD4$^+$ T cells exhibiting an unbalanced Th1-type cell cytokine pattern: reversal with IL-10. *J. Immunol.* 160: 3602-3609, 1998.

Langford, C.A., Talar-Williams, C., Barron, K.S., and Sneller, M.C. A staged therapeutic approach in the treatment of Wegener's granulomatosis: induction with glucocorticoids and daily cyclophosphamide switching to methotrexate for remission maintenance. *Arthritis Rheum.* 42:2666-2673, 1999.

Langford, C.A., Talar-Williams, C., and Sneller, M.C. Use of methotrexate and glucocorticoids in the treatment of Wegener's granulomatosis: long-term renal outcome in patients with glomerulonephritis. *Arthritis Rheum.* 43:1836-1840, 2000.

HIV/AIDS Clinical Research Program

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Judith Falloon, M.D.
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Dr. Davey received his M.D. from Columbia Univ., trained in internal medicine at Boston Univ. Hospital and in infectious diseases at NIAID, and joined the intramural AIDS program in 1987. Dr. Falloon received her M.D. from the Univ. of Maryland in 1980, trained in internal medicine at Washington Univ. and in infectious diseases at NIAID, and joined the intramural AIDS program in 1988. Dr. Polis obtained his M.D. from the Albert Einstein College of Medicine, his M.P.H. at the Johns Hopkins School of Public Health, trained in internal medicine and infectious diseases at the George Washington Univ. Medical Center and in epidemiology at NIAID. He joined the intramural AIDS program in 1988.

Description of Research Program

The HIV/AIDS clinical research program of the intramural AIDS program has as its mission the completion of innovative, high quality clinical research in the areas of HIV/AIDS therapeutics and pathogenesis. The trials are conducted in a multi-disciplinary clinic staffed by senior and junior level physicians, research nurses, nurse case managers, pharmacists, laboratory and computer support staff, and social workers. Our work also depends on the patient volunteers who enroll in our trials and studies. The portfolio of open trials and studies changes frequently.

Major Areas of Research

- Treatments for HIV infection and its consequences including immune-based therapies and treatment interruption strategies
- Studies of immune function, immunodeficiency and the pathogenesis of HIV disease
- Investigations into the complications of HIV infection or its treatment including opportunistic infections, hepatitis B and C, and drug toxicities

Selected Recent Publications

Davey Jr., R.T., Bhat, N., Yoder, C., *et al.* HIV-1 and T Cell dynamics following interruption of HAART in patients with a history of sustained viral suppression. *Proc. Natl. Acad. Sci. USA* 96:15109-114, 1999.

Davey Jr., R.T., Murphy, R., Graziano, F., *et al.* Immunologic and virologic effects of subcutaneous interleukin 2 in combination with antiretroviral therapy: A randomized clinical trial. *JAMA* 284:183-189, 2000.

Hatano, H, Miller, K.D., Yoder, C.P., Yanovski, J.A., Sebring, N.G., Jones, E.C., Davey, R.T. Jr. Metabolic and anthropometric consequences of interruption of highly active antiretroviral therapy. *AIDS* 14:1935-42, 2000.

Piscitelli, S.C., Burstein, A.H., Chaitt, D., Alfaro, R.M., Falloon, J. Indinavir concentrations and St. John's wort. *Lancet* 355: 547-8, 2000.

Falloon, J. *et al.* Combination therapy with amprenavir, abacavir and efavirenz in human immunodeficiency virus (HIV)-infected patients failing a protease-inhibitor regimen: pharmacokinetic drug interactions and antiviral activity. *Clin. Infect. Dis.* 30:313-8, 2000.

Whitcup, S.M., *et al.* Discontinuation of anticytomegalovirus therapy for patients with HIV infection and cytomegalovirus retinitis. *JAMA* 282:1633-7, 1999.

Hatano, H., *et al.* Pre-HAART HIV burden approximates post-HAART viral levels following interruption of therapy in patients with sustained viral suppression. *AIDS* 14:1357-63, 2000.

Laboratory of Infectious Diseases

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Suzanne U. Emerson, Ph.D., Head

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Elvera Ehrenfeld, Ph.D., Head

Research Activities

Studies in the Laboratory of Infectious Diseases (LID) focus primarily on viruses that play an important role in disease of the respiratory and gastrointestinal tracts, the liver, and the reticuloendothelial system. Currently, LID employs the techniques of viral genetics and molecular biology to express protective viral antigens and to attenuate viral mutants that may prove useful for prevention of disease. A number of promising subunit and live attenuated viral vaccines and virus-specific immunotherapies are under development and are being evaluated in animals and humans.

The LID conducts research directed toward defining the cause and epidemiology of medically important viral diseases and developing means for their control. LID engages in a wide range of research activities that extends from identification and antigenic characterization of viruses that cause systemic disease or acute disease of the respiratory and gastrointestinal tracts and the liver to basic molecular studies of viral structure, function, and genome organization. Scientists in the laboratory employ molecular biologic techniques to elucidate pathogenesis of disease, to determine the major mediators of immunity to the viruses under study, and to develop purified subunit antigens and attenuated viral mutants for use in prevention of respiratory, gastrointestinal, hepatic, and systemic viral diseases. The major viruses under study include hepatitis A-E viruses, dengue viruses, tick-borne encephalitis and West Nile viruses, rotaviruses, caliciviruses, respiratory syncytial virus, parainfluenza viruses, measles viruses, and influenza viruses.

Brian Murphy, M.D.

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Dr. Murphy received his M.D in 1968 from University of Rochester and did an internship at Stanford University before joining NIH in 1970. He has headed the Respiratory Viruses Section since 1983. His research focuses on understanding the immunobiology and molecular biology of influenza viruses, parainfluenza viruses, respiratory syncytial viruses, and dengue viruses, and on the development of vaccines to prevent diseases caused by these viruses. He serves on the editorial boards of *Virology* and *Journal of Virology*.

Description of Research Program

The research program of the Respiratory Viruses Section aims at the development of vaccines for several important viral pathogens and encompasses a spectrum of research activities including recovering infectious viruses from cDNAs, producing a variety of attenuating mutations, inserting such mutations into wild type or incompletely attenuated viruses, evaluating the effect of the introduced mutation on replication and pathogenesis of the mutant viruses in rodents or non-human primates, determining the genes of the virus under study that induce protective immune responses, preparing experimental lots of vaccines for administration to humans, and evaluating the experimental vaccines in humans. The techniques of molecular virology are used to attenuate viruses, which include respiratory syncytial virus, the human parainfluenza viruses, flaviviruses including dengue and tick borne-encephalitis viruses, and measles virus. The effect of an introduced mutation on replication of the mutant viruses is studied in tissue cultures, animals, non-human primates, and humans, as well in the mosquito vectors of the dengue viruses.

Major Areas of Research

- Recover the human parainfluenza type 1, 2, and 3 viruses from cDNA and develop sets of attenuating mutations that permit efficient replication in tissue culture but restricted replication *in vivo.* Use PIV viruses as vectors of other viral antigens such as RSV and measles virus.
- Recover dengue viruses types 1, 2, 3, and 4 from cDNA and develop mutations that restrict replication in mosquitoes, human liver cells, and in monkeys and humans. Produce antigenic chimeric viruses bearing the structural proteins of dengue type 1, 2 or 3 on an attenuated dengue 4 backbone.
- Develop attenuated mutants of tick-borne encephalitis virus.
- Collaborate with Dr. Collins to develop satisfactorily attenuated RSV vaccine candidates that are genetically altered to induce high levels of protective immune responses.

Selected Recent Publications

Bailly, J.E., McAuliffe, J.M., Durbin, A.P., Elkins, W.R., Collins, P.L., Murphy, B.R. A recombinant human parainfluenza virus type 3 (PIV3) in which the nucleoscapsid N protein has been replaced by that of bovine PIV3 is attenuated in primates. *J. Virol.* 74: 3188-95, 2000.

Durbin, A.P., Skiadopoulos, M.H., McAuliffe, J.M., Riggs, J.M., Surman, S.R., Collins, P.L., Murphy, B.R. Human parainfluenza type 3 (PIV3) expressing the hemagglutinin protein of measles virus provides a novel method for immunization against measles virus and PIV3 in early infancy. *J. Virol.* 74: 6821-31, 2000.

Wright, P.F., Karron, R.A., Belshe, R.B., Thompson, J., Crowe, J.E. Jr., Boyce, T.G., Halburnt, L.L., Reed, G.W., Whitehead, S.S., Anderson, E.L., Wittek, A.E., Casey, R., Eichelberger, M., Thumar, B., Randolph, V.B., Udem, S.A., Chanock, R.M., Murphy, B.R. Evaluation of a cold-passaged, temperature-sensitive, respiratory syncytial virus (RSV) vaccine candidate in infancy. *J. Infect. Dis.* 182: 1331-42, 2000.

Robert H. Purcell, M.D.

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Dr. Purcell obtained a master's degree in biochemistry from Baylor University and a medical degree and pediatric internship at Duke University and Hospital. His research focuses on the hepatitis viruses, with special emphasis on their molecular biology, epidemiology, and control. He is the author or coauthor of over 600 publications, and is a member of the National Academy of Sciences.

Description of Research Program

The Hepatitis Viruses Section conducts basic research on the hepatitis viruses and applies the knowledge gained to the control of viral hepatitis. It has contributed to the development of licensed vaccines for hepatitis A and hepatitis B, to a candidate vaccine for hepatitis E and to the discovery of hepatitis C and hepatitis D. Current research efforts include the elucidation of the molecular virology of hepatitis A and the development of a live-attenuated hepatitis A vaccine, delineation of the genetic heterogeneity of hepatitis C virus, diagnosis and prevention of hepatitis E, and molecular and biological characterization of newly discovered viruses.

Major Areas of Research

- Seroepidemiology and molecular epidemiology
- Pathogenesis and animal models of human disease
- Active and passive immunoprophylaxis
- Discovery of new viruses

Selected Recent Publications

Tsarev, S.A., Tsareva, T.S., Emerson, S.U., Govindarajan, S., Shapiro, M., Gerin, J.L., Purcell, R.H. Recombinant vaccine against hepatitis E: dose response and protection against heterologous challenge. *Vaccine* 15:1834-1838, 1997.

Ogata, N., Cote, P.J., Zanetti, A.R., Miller, R.H., Shapiro, M., Gerin, J., Purcell, R.H. Licensed recombinant hepatitis B vaccines protect chimpanzees against infection with the prototype surface gene mutant of hepatitis B virus. *Hepatology* 30: 779-786, 1999.

Farci, P., Shimoda, A., Coiana, A., Melpolder, J.C., Peddis, G., Strazzera, A., Chien, D.Y., Balestrieri, A., Purcell, R.H., Alter, H.J. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science* 288:339-344, 2000.

Forns, X., Thimme, R., Govindarajan, S., Emerson, S.U., Purcell, R.H., Chisari, F.V., Bukh, J. Hepatitis C virus lacking the hypervariable region 1 of the second envelope protein is infectious and causes acute resolving or persistent infection in chimpanzees. *Proc. Natl. Acad. Sci. USA* 97:13318-13323, 2000.

Forns, X., Payette, P.J., Ma, X., Satterfield, W., Eder, G., Mushahwar, I.K., Govindarajan, S., Davis, H.L., Emerson, S.U., Purcell, R.H., Bukh, J. Vaccination of chimpanzees with plasmid DNA encoding the hepatitis C virus (HCV) envelope E2 protein modified the infection after challenge with homologous monoclonal HCV. *Hepatology* 32: 618-25, 2000.

Bukh, J., Apgar, C.L., Govindarajan, S., Emerson, S.U., Purcell, R.H. Failure to infect rhesus monkeys with hepatitis C virus strains of genotypes 1a, 2a or 3a. *J. Viral Hepat.* 8:228-31, 2001.

Jens Bukh, M.D.

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Dr. Bukh obtained a medical degree at the University of Copenhagen, Denmark. His research focuses on the hepatitis C virus and related viruses. Dr. Bukh is the author or coauthor of over 50 publications, and is a member of the American Association for the Study of Liver Diseases. He serves on the editorial board of *Journal of Clinical Microbiology.*

Description of Research Program

As a member of the Hepatitis Viruses Section, Dr. Bukh has conducted basic research on hepatitis C virus and the related GB viruses (GB virus A, GB virus B and GB virus C). This research has contributed to the characterization of genetic heterogeneity, to the development of infectious molecular clones and to a better understanding of the natural history of these viruses. Current research efforts include the elucidation of the molecular virology of hepatitis C virus and GB virus B and their natural history in primate animal models, as well as the development of DNA-based vaccine candidates for HCV.

Major Areas of Research

- Molecular virology of hepatitis C virus and related viruses
- Immunoprophylaxis against hepatitis C virus

Selected Recent Publications

Bukh, J., Apgar, C.L., Yanagi, M. Toward a surrogate model for hepatitis C virus: An infectious molecular clone of the GB virus-B hepatitis agent. *Virology* 262:470-478, 1999.

Yanagi, M., St. Claire, M., Emerson, S.U., Purcell, R.H., Bukh, J. In vivo analysis of the 3' untranslated region of hepatitis C virus following *in vitro* mutagenesis of an infectious cDNA clone. *Proc. Natl. Acad. Sci. USA* 96:2291-2295, 1999.

Forns, X., Thimme, R., Govindarajan, S., Emerson, S.U., Purcell, R.H., Chisari, F.V., Bukh, J. Hepatitis C virus lacking the hypervariable region 1 of the second envelope protein is infectious and causes acute resolving or persistent infection in chimpanzees. *Proc. Natl. Acad. Sci. USA* 97:13318-13323, 2000.

Allander, T., Forns, X., Emerson, S.U., Purcell, R.H., Bukh, J. Hepatitis C virus envelope protein E2 binds to CD81 of tamarins. *Virology* 277: 358-67, 2000.

Forns, X., Payette, P.J., Ma, X., Satterfield, W., Eder, G., Mushahwar, I.K., Govindarajan, S., Davis, H.L., Emerson, S.U., Purcell, R.H., Bukh, J. Vaccination of chimpanzees with plasmid DNA encoding the hepatitis C virus (HCV) envelope E2 protein modified the infection after challenge with homologous monoclonal HCV. *Hepatology* 32: 618-25, 2000.

Bukh, J., Apgar, C.L., Govindarajan, S., Emerson, S.U., Purcell, R.H. Failure to infect rhesus monkeys with hepatitis C virus strains of genotypes 1a, 2a or 3a. *J. Viral Hepat.* 8:228-31, 2001.

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Dr. Chanock received his M.D. in 1947 and Honorary Doctor of Science in 1977 from University of Chicago. He became a member of the U.S. National Academy of Sciences in 1973 and the Danish Royal Society of Sciences in 1980. He is a recipient of the Robert Koch Medal, ICN International prize in Virology, and Bristol-Meyers Squibb Award for Distinguished Achievement in Infectious Diseases.

Description of Research Program

Studies focus on molecular cloning of human monoclonal antibodies that neutralize important respiratory tract viruses such as respiratory syncytial virus (RSV) and evaluation of their usefulness in passive immunoprophylaxis or immunotherapy. Participation in LID programs for the derivation and evaluation of live attenuated virus vaccines for prevention of serious disease that is systemic (dengue) or primarily localized in the respiratory tract (RSV and parainfluenza viruses) or gastrointestinal tract (rotaviruses) is a research priority. Vaccines are derived by biological manipulation or chemical mutagenesis, recombinant DNA technology, or a combination of both.

Surveillance of virus vaccines developed by LID scientists during the post-licensure period and collaboration with other scientists in the laboratory to address issues of safety and efficacy of the live attenuated rotavirus vaccine developed in LID are additional areas of focus. Other workers had described an increased risk of intussusception associated with the vaccine based on analysis of a short-term study of a small population. However, analysis of a large population-based epidemiologic study of one million infants during their first year of life failed to detect any attributable risk.

Dr. Chanock collaborates as a consultant with organizations outside NIH to resurrect and restore a safe, highly effective, live adenovirus vaccine developed 30 years ago in LID. This vaccine, licensed 20 years ago, routinely prevented outbreaks of adenovirus disease in the military, which have now returned in force following cessation of production by the manufacturer. Fortunately, the military is seeking a new manufacturer but it will be necessary to revalidate vaccine formulation, vaccine production and clinical evidence of safety and protective efficacy again, a process that will require 4 to 5 years. Dr. Chanock will serve as a consultant during this interval to provide expertise in these activities. In addition, attempts will be made to extend the usefulness of the live attenuated adenovirus vaccine approach to other populations at high risk.

Major Areas of Research

- Molecular biology of single- or double-stranded RNA viruses
- Pathogenesis of disease
- Development of vaccines for important human viruses
- Passive protection or therapy by human monoclonal antibodies

Selected Recent Publications

Sakurai, H., Williamson, R.A., Crowe, J.E., Beeler, J.A., Poignard, P., Bastidas, R.B., Chanock, R.M., Burton, D.R. Human antibody responses to mature and immature forms of viral envelope in respiratory syncytial virus infection: significance for subunit vaccines. *J. Virol.* 73: 2956-62, 1999.

Wright, P.F., Karron, R.A., Belshe, R.B., Thompson, J., Crowe, J.E. Jr., Boyce, T.G., Halburnt, L.L., Reed, G.W., Whitehead, S.S., Anderson, E.L., Wittek, A.E., Casey, R., Eichelberger, M., Thumar, B., Randolph, V.B., Udem, S.A., Chanock, R.M., Murphy, B.R. Evaluation of a cold-passaged, temperature-sensitive, respiratory syncytial virus (RSV) vaccine candidate in infancy. *J. Infect. Dis.* 182: 1331-2, 2000.

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Dr. Collins received a Ph.D. in 1981 from University of Connecticut studying microbiology. He then conducted postdoctoral research at the University of North Carolina, where he achieved the molecular cloning and sequencing of respiratory syncytial virus. In 1984 he joined the LID and received tenure in 1990. He serves on the editorial board of *Virology.*

Description of Research Program

Acute respiratory tract infection is the leading cause of death worldwide due to infectious disease. Respiratory syncytial virus (RSV) is among the most important agents of acute respiratory tract infection worldwide, and is the most important viral respiratory pathogen in the pediatric population. RSV is an enveloped virus with a nonsegmented negative-sense RNA genome. We have a long-standing program to study RSV molecular biology and use this information to understand viral pathogenesis and to develop vaccines for the pediatric and adult populations. One type of study involves reconstituting viral activities such as encapsidation, transcription, RNA replication and virion morphogenesis from components expressed from cloned cDNAs. This provides a method for introducing predetermined changes into viral proteins and cis-acting RNA signals to characterize these viral activities. Another approach is to recover complete infectious recombinant virus from cDNA, which provides a method for systematically altering the virus in order to characterize viral replication *in vitro* and *in vivo*, viral pathogenesis, and the host immune response. This also provides a method for making engineered live attenuated vaccine virus. A number of candidate vaccine viruses are in the process of preclinical and clinical evaluation. These studies are performed in close collaboration with Dr. Brian R. Murphy, and comparable studies are also being performed with parainfluenza viruses types 1, 2 and 3, which also are important etiologic agents of respiratory tract disease worldwide. The development of highly characterized, engineered, cDNA-derived viruses for widespread vaccination represents a new phase in vaccine research.

Major Areas of Research

- Viral molecular biology
- Viral pathogenesis
- Vaccine development
- RNA viruses

Selected Recent Publications

Bermingham, A., Collins, P.L. The M2-2 protein of human respiratory syncytial virus is a regulatory factor involved in the balance between RNA replication and transcription. *Proc. Natl. Acad. Sci. USA* 96:1259-11264, 1999.

Bukreyev, A., Whitehead, S.S., Bukreyeva, N., Murphy, B.R., Collins, P.L. Interferon gamma expressed by a recombinant respiratory syncytial virus attenuates virus replication in mice without compromising immunogenicity. *Proc. Natl. Acad. Sci. USA* 96:2367-72, 1999.

Buchholz, U.J., Schuldt, K., Granzow, H., Whitehead, S.S., Murphy, B.R., Collins, P.L. Chimeric bovine respiratory syncytial virus (BRSV) with glycoprotein gene substitutions from human respiratory syncytial virus (HRSV): effects on host range and evaluation as a live-attenuated HRSV vaccine. *J. Virol.* 74:1187-1199, 2000.

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Dr. Ehrenfeld obtained her Ph.D. in biochemistry at the University of Florida. During postdoctoral work at Albert Einstein College of Medicine, she began studies of molecular aspects of poliovirus replication in cultured human cells. She joined the faculty at Einstein, then continued her research and teaching career at the University of Utah School of Medicine and subsequently at the University of California, Irvine, where she also served as Dean of the School of Biological Sciences. In 1997, Dr. Ehrenfeld came to NIH as Director, Center for Scientific Review, and joined the Laboratory of Viral Diseases in NIAID as Head of the Picornavirus Replication Section. Her research focuses on mechanisms of RNA replication and regulation of protein synthesis in poliovirus- and hepatitis A virus-infected cells. She has served on numerous editorial boards and consulted for numerous academic, government, and private sector agencies. She is the recipient of honors and awards for research and teaching in virology and molecular biology.

Description of Research Program

Our laboratory studies the molecular mechanisms of several aspects of the replication of picornaviruses. This virus family includes numerous human pathogens (poliovirus, Coxsackievirus, echovirus, enteroviruses, rhinoviruses, hepatitis A virus). Infection of cells with these viruses leads to major changes in the host cell structure and metabolic activity. Cellular protein and RNA synthesis are inhibited; the intracellular membrane network becomes rearranged into vesicles that surround and provide a scaffold for viral RNA replication complexes; cellular proteins are subverted into facilitating viral protein and RNA synthesis. The unique combination of viral and cellular proteins accomplishes highly efficient production of viral RNA, proteins, and particles.

We are studying the activities of individual viral gene products, expressed alone or in combination, in cultured human cells, to determine their individual roles in the replication process. The abilities of individual viral proteins to induce cellular membrane rearrangements have been determined. We have developed biochemical assays for specific steps in the RNA replication reaction to elucidate the requirements and mechanisms for each step. We have engineered or identified mutations that generate viral proteins that are defective in RNA synthesis. These reagents allow us to determine the relationship between viral protein and RNA structures and their biochemical functions. The interactions of each viral protein with each other, with critical regions of the viral RNA, and with cellular proteins are being characterized. Ultimately, we hope to understand and reconstruct each step of the replication reaction *in vitro.*

Major Areas of Research

- RNA replication
- RNA and protein structure/function
- Virus - host cell interactions

Selected Recent Publications

Bell, Y., Semler, B.L., Ehrenfeld, E. Requirements for RNA replication of a poliovirus replicon by Coxsackievirus B3 RNA polymerase. *J. Virol.* 73: 9413-9421, 1999.

Egger, D., Teterina, N., Ehrenfeld, E., Bienz, K.Ü. The formation of the poliovirus replication complex requires coupled viral translation vesicle production and viral RNA synthesis. *J. Virol.* 74: 6570-6580, 2000.

Teterina, N., Egger, D., Bienz, K., Brown, D., Semler, B., Ehrenfeld, E. Requirements for assembly of poliovirus replication complexes and negative strand RNA synthesis. *J. Virol.* 75: 3841-3850, 2001.

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Dr. Emerson obtained her doctorate from the University of California, San Diego, for the study of assembly of bacterial flagella. Following postdoctoral research at the University of Virginia Medical School, she joined the faculty of the Department of Microbiology where she carried out molecular studies on vesicular stomatitis virus. She joined the Hepatitis Viruses Section of LID in 1988 and in 1998 became Section Head of the newly created Molecular Hepatitis Section. Her research focuses on basic molecular studies of the hepatitis viruses A, E, and C and on the development of vaccines for these viruses.

Description of Research Program

The molecular biology of hepatitis A virus is being studied by constructing chimeric viruses from infectious cDNA clones of viruses with different phenotypes. The phenotype of each mutant is determined in cell culture and the natural history is determined following infection or transfection of nonhuman primates. Challenge studies are performed in nonhuman primates in order to evaluate attenuated vaccine candidates. Hepatitis E virus has not been successfully grown in cell culture so natural history determinations and vaccine evaluations are done in nonhuman primates. An infectious cDNA is being constructed that will be used to study the molecular biology and host range of the hepatitis E virus. The hepatitis E virus circulating in wild rats in the U.S. is being passaged in laboratory rats and will be characterized and its importance as a zoonosis will be evaluated. The efficacy of recombinant vaccine candidates is being assessed.

Major Areas of Research

- Vaccines for hepatitis viruses
- Molecular biology of hepatitis A virus and hepatitis E virus

Selected Recent Publications

Meng, X.J., Halbur, P.G., Shapiro, M.S., Govindarajan, S., Bruna, J.D., Mushahwar, I.K., Purcell, R.H., Emerson, S.U. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J. Virol.* 72: 9714-9721, 1998.

Raychaudhuri, G., Govindarajan, S., Shapiro, M., Purcell, R.H., Emerson, S.U. Utilization of chimeras between human (HM-175) and simian (AGM-27) strains of hepatitis A virus to study the molecular basis of virulence. *J. Virol.* 72: 7467-7475, 1998.

Kabrane-Lazizi, Y., Meng, X.-J., Purcell, R.H., Emerson, S.U. Evidence that the genomic RNA of hepatitis E virus is capped. *J. Virol.* 73: 8848-8850, 1999.

Kabrane-Lazizi, Y., Zhang, M., Purcell, R.H., Miller, K.D., Davey, R.T., Emerson, S.U. Acute hepatitis caused by a novel strain of hepatitis E virus most closely related to United States strains. *J. Gen. Virol.* 82:1687-93, 2001.

Purcell, R.H., Emerson, S.U. Animal models of hepatitis A and E. *ILAR J.* 42:161-77, 2001.

Yasutaka Hoshino, D.V.M.

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Dr. Hoshino received his doctoral degree in veterinary medicine from Nihon University, Tokyo, Japan, studying virology.

Description of Research Program

Rotaviruses are consistently found to be the single most important causative agents of severe diarrhea in infants and young children worldwide. Rotavirus diarrhea is estimated to cause approximately 800,000 deaths every year in developing countries, which is equivalent to 25% of all diarrheal deaths and 6% of all deaths in children less than 5 years of age. In the United States alone, rotavirus diarrhea is estimated to be responsible for more than 500,000 physician visits, 50,000 hospitalizations and over $1 billion of societal expenses each year. Therefore, the development of a safe and effective rotavirus vaccine has been a high priority public health goal endorsed by various organizations including the World Health Organization.

In many viral diseases, neutralizing antibodies appear to play an important role in protection against disease and/or infection in a type-specific manner. Thus, rotavirus antigens that can induce neutralizing antibodies have played a central role in research and development of a rotavirus vaccine. The outer capsid of rotaviruses is composed of two proteins, VP4 and VP7, both of which are distinct neutralization antigens. One of our projects is to characterize selected human and animal rotavirus strains isolated from various geographical areas and special situations in order to define the extent of VP4 and VP7 polymorphism. Such studies may have important implications for the development of effective rotavirus vaccine candidates. Another project is to identify the rotaviral genes that play a major role in rotavirus virulence or host range restriction and attenuation and apply information obtained from such studies to the formulation of a strategy for the development of safe and effective rotavirus vaccines that are of optimal efficacy. Genetic and molecular analyses of various rotavirus reassortants as well as cold-adapted rotavirus vaccine candidates are underway. We have established various animal models to study rotavirus pathogenesis and immunity including gnotobiotic piglets, rhesus monkeys and rat pups in which various candidates vaccines (both live and non-viable) have been tested. Generation of rotavirus specific human recombinant neutralizing monoclonal antibodies of therapeutic potential is our new project.

Major Areas of Research

- Rotavirus vaccine development
- Rotavirus pathogenesis and immunity
- Rotavirus VP7 and VP4 serotypes/genotypes

Selected Recent Publications

Hoshino, Y., Jones, R.W., Chanock, R.M., Kapikian, A.Z. Construction of four double gene substitution human x bovine rotavirus reassortant vaccine candidates: each bears two outer capsid human rotavirus genes, one encoding P serotype 1A and the other encoding G serotype 1, 2, 3, or 4 specificity. *J. Med. Virol.* 51: 319-325, 1997.

Hoshino, Y., Kapikian, A.Z. Rotavirus serotypes: classification and importance in rotavirus epidemiology, immunity and vaccine development. *J. Health Popul. Nutr.* 18: 5-14, 2000.

Santos, N., Volotao, E.M., Soares, C.C., Albuquerque, M.C.M., DaSilva, F.M., DeCarvalho, T.R.B., Pereira, C.F.A., Chizhikov, V., Hoshino, Y. Rotavirus strains bearing genotype G9 or P[9] recovered from Brazilian children with diarrhea from 1997 to 1999. *J. Clin. Microbiol.* 39:1157-1160, 2001.

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Dr. Kapikian received his M.D. from Weill Medical College of Cornell University (formerly Cornell University Medical College) in 1956.

Description of Research Program

The goals of the Epidemiology Section are to study the natural history and epidemiology of acute illness and infection; discover new infectious agents and determine their etiologic role in disease; characterize such agents by virologic and molecular biologic techniques; and determine methods for disease control with special emphasis on vaccines. Immune electron microscopy is utilized as a method for detection, identification, and characterization of fastidious agents of viral gastroenteritis and other illnesses.

We discovered the first major viral gastroenteritis agent of adults–the Norwalk virus, and continue to study the epidemiology of Norwalk and Norwalk-like viruses in various population groups. We also study the epidemiology and characteristics of the rotaviruses, which were discovered in Australia, and have emerged as the single most important cause of diarrhea of infants and young children worldwide, responsible for 600,000 to 800,000 deaths in the developing countries in this age group. We developed an effective rotavirus vaccine which was licensed by the FDA in 1998 and which was given to about one million infants. However, the vaccine was withdrawn when a rare adverse event—intussusception—was associated with it. We are presently attempting to learn the overall attributable risk, if any, of this rare event. Several second-generation vaccines have been developed but we are awaiting the final decision regarding the initial rotavirus vaccine.

Major Areas of Research

- Viral gastroenteritis
- Electron microscopy
- Vaccine development

Selected Recent Publications

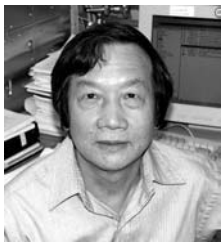
Perez, M.E., Glass, R., Alvarez, G., Pericchi, L.R., Gonzalez, R., Kapikian, A.Z., Perez-Schael, I. Rhesus rotavirus-based quadrivalent vaccine is efficacious despite age, socioeconomic conditions and seasonality in Venezuela. *Vaccine* 19: 976-981, 2001.

Kapikian, A.Z. A rotavirus vaccine for prevention of severe diarrhea of infants and young children: development, utilization and withdrawal. In: Novartis Foundation Symposium 263. Gastroenteritis Viruses. Chichester, U.K.: John Wiley and Sons, Ltd., 2001, p. 153-179.

Clements-Mann, M.L., Dudas, R., Hoshino, Y., Nehring, P., Sperber, E., Wagner, M., Stephens, I., Karron, R., Deforest, A., Kapikian, A.Z. Safety and immunogenicity of live attenuated quadrivalent human-bovine (U.K.) reassortant rotavirus vaccine administered with childhood vaccines to infants. *Vaccine* 9: 4676-84, 2001.

Hoshino, Y., Kapikian, A.Z. Rotavirus serotypes: classification and importance in epidemiology, immunity, and vaccine development. *J. Health Popul. Nutr.* 18: 5-14, 2000.

Kapikian, A.Z. The discovery of the 27-nm Norwalk virus: an historic perspective. *J. Infect.Dis.* 181 Suppl. 2: S295-302, 2000.



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Dr. Lai received his Ph. D. in 1972 in molecular biology from the University of Wisconsin, Madison, studying erythromycin-inducible resistance of *Staphylococcus aureus.* His subsequent research areas included functional mapping of SV40 DNA using restriction enzymes and the organization of the influenza virus segmented genome by cDNA cloning, sequencing and expression of the gene segments in mammalian cells. Dr. Lai serves on the editorial board of *Virology.*

Description of Research Program

Dengue epidemics caused by one or more of the four dengue virus serotypes increasingly pose a major public health problem in most countries of the tropical and subtropical regions, where the mosquito vectors are abundant. A severe form of dengue disease that has a high mortality rate, characterized by dengue hemorrhagic fever and dengue hemorrhagic fever with shock syndrome, may occur in young children. A safe and effective vaccine against dengue virus infection is still not available. Studies are focused on dengue viruses and other emerging insect-borne related flaviviruses, including Japanese encephalitis virus and tick-borne encephalitis virus, to develop immunoprophylactic strategies against these viruses. Defined mutations are introduced into dengue type 4 virus cDNA and its derived chimeras of other serotypes to investigate the molecular mechanisms that are responsible for reduced viral replication in cultured cells and attenuation of virulence in animal models and in humans. Stable mutants that exhibit a reduced efficiency of viral replication in cell culture should also prove valuable for evaluation of attenuation phenotype. We also analyze the antigenic structures and design strategies to increase the immunogenicity and protective efficacy of the major dengue virus antigens in animal models. Recently, we have been using phage display of combinatorial antibody libraries to identify dengue virus-specific monoclonal antibodies from chimpanzees (or humans) infected with one or more of the four dengue virus serotypes. The goal is to identify chimpanzee or human antibodies highly effective in neutralizing dengue viruses that could be used in passive immunization for prevention against dengue virus infection in humans. As adverse reactions are very rare when humans are administered the large amount of antibodies required to reach the physiologic level, such antibodies might also be considered as an effective therapy for severe dengue.

Major Areas of Research

- Identification of dengue virus neutralizing monoclonal antibody Fab fragments and their derived whole IgG molecules from chimpanzees or humans by repertoire cloning for potential use in passive prophylaxis and /or therapy
- Analysis of flavivirus antigenic structures replicating or non-replicating and development of vaccine strategies
- Analysis of genetic determinants responsible for dengue virulence in animal models and dengue pathogenesis in humans

Selected Recent Publications

Bray, M., Men, R., Tokimatsu, I., Lai, C.-J. Genetic determinants responsible for acquisition of dengue type 2 mouse neurovirulence. *J. Virol.* 72: 1647-1651, 1998.

Men, R., Wyatt, L., Tokimatsu, I., Arakaki, S., Shameem, G., Elkins, R., Chanock, R., Moss, B., Lai, C.-J. Immunization of rhesus monkeys with a recombinant of modified vaccinia virus Ankara expressing a truncated envelope glycoprotein of dengue type 2 virus induced resistance to dengue type 2 virus challenge. *Vaccine* 18:3113-3122, 2000.

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Dr. Patton received his Ph.D. in 1980 from Virginia Polytechnic Institute for his work on the molecular biology of the parvoviruses. Following postdoctoral research at the University of North Carolina Medical School, Chapel Hill, on the replication of vesicular stomatitis virus, he joined the faculty at the University of South Florida. In 1987, Dr. Patton moved to the University of Miami School of Medicine where he remained until 1996 when he took a position in the Laboratory of Infectious Diseases

Description of Research Program

Among the families of viruses, the Reoviridae are distinct, containing those with genomes made up of several segments of double-stranded (ds) RNA. Some of the members of this family are significant pathogens of animals, both vertebrates and invertebrates, and plants. One member, the rotaviruses, are the most important cause of acute severe diarrhea in infants and young children and, each day, are responsible for nearly 3000 deaths throughout the world. The research goals of the laboratory are to describe the replication process of the rotaviruses, define the role of the viral proteins in modulating host cell pathways and immune responses, and develop effective rotavirus vaccines. Multiple approaches are being used to address these goals including the study of the structural and functional properties of recombinant viral proteins, analysis of the packaging and replication of the viral genome in cell-free systems, manipulation of the viral genome by reverse genetics, and characterization of infectious viruses in cell culture and animal model systems.

Major Areas of Research

- RNA viruses
- Protein structure and function
- Viral genome packaging and replication
- Vaccine development

Selected Recent Publications

Patton, J.T., and E. Spencer. Mini-review: Genome replication and packaging of segmented double-stranded RNA viruses. *Virology* 277: 217-225, 2000.

Chizhikov, V., and J.T. Patton. A four-nucleotide translation enhancer in the 3′-terminal consensus sequence of the nonpolyadenylated mRNAs of rotavirus. *RNA* 6: 814-825, 2000.

Chen, D., and J.T. Patton. *De novo* synthesis of minus-strand RNA by the rotavirus RNA polymerase in a cell-free system involves a novel mechanism of initiation. *RNA* 6: 1455-1467, 2000.

Patton, J.T. Rotavirus RNA replication and gene expression. In: Novartis Foundation Symposium 238. Gastroenteritis Viruses. Chichester, U.K.: John Wiley and Sons, Ltd., 2001, p. 64-77.

Schuck, P., Z. Taraporewala, P. McPhee, and J.T. Patton. The nonstructural protein NSP2 of rotavirus forms an octamer that undergoes ligand-induced structural changes. *J. Biol. Chem.* 276: 9679-9687, 2001.

Patton, J.T., Z. Taraporewala, D. Chen, V. Chizhikov, M. Jones, A. Elhelu, M. Collins, K. Kearney, M. Wagner, Y. Hoshino, and V. Gouvea. Effect of intragenic rearrangement and changes in the 3′-consensus sequence on NSP1 expression and rotavirus replication. *J. Virol.* 75: 2076-2086, 2001.

Taraporewala, Z., and J.T. Patton. Identification and characterization of the helix destabilizing activity of the rotavirus nonstructural protein NSP2. *J. Virol.* 75: 4519-4527, 2001.

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Dr. Pletnev received his Ph.D. in 1983 in chemistry from the Academy of Sciences of the USSR, studying RNA polymerases. Following postdoctoral research at Novosibirsk Institute of Organic Chemistry of the Academy of Sciences and teaching at the Novosibirsk State University, he was Chief of the Laboratory of Molecular Virology from 1984 to 1993. He joined LID in 1993 and became a Principal Investigator in 1997.

Description of Research Program

The tick-borne flavivirus complex of the *Flaviviridae* family that includes Kyasanur forest disease, Louping ill, Negishi, Omsk hemorrhagic fever, tick-borne encephalitis and Powassan viruses are endemic throughout most of the northern hemisphere and cause human disease of varying severity that can have a mortality as high as 30%. Tick-borne encephalitis caused by viruses of this complex remains a pressing public health problem in Europe and Asia, where 9,000-12,000 patients are diagnosed annually. Currently, a vaccine produced by formalin inactivation of tick-borne encephalitis virus (TBEV) is available, but multiple inoculations are required to achieve a satisfactory level of resistance and the breadth of its protective effect has been questioned.

We developed a new approach to immunoprophylaxis, which involved the construction of recombinant chimeric flaviviruses that contain the genes for the structural premembrane and envelope proteins of TBEV or Langat virus (LGT) with the rest of the genome derived from dengue type 4 virus (DEN4). The resulting LGT/DEN4 or TBEV/DEN4 chimera exhibited a modest reduction in neurovirulence for Swiss mice, as tested by intracerebral inoculation. More impressive was the effect of chimerization on neuroinvasiveness, i.e., the ability of virus to spread from peripheral tissues to the central nervous system where it produces fatal encephalitis. Chimerization of TBEV or LGT with DEN4 completely ablated detectable neuroinvasiveness when assayed by the most sensitive indicator system available, the immunodeficient (SCID) mouse. Significantly, these chimeras provided protection in immunocompetent mice against LGT as well as the highly virulent TBEV of both the European subtype and the Far Eastern subtype. More recently we observed that immunization with the LGT/DEN4 chimera provided complete protection when immunized monkeys were challenged subcutaneously with the wild-type LGT strain TP21. These observations offer encouragement for the successful development of a live attenuated TBEV vaccine. Also, this provides a strategy for constructing attenuated chimeric viruses bearing the protective antigens of various highly virulent tick-borne or mosquito-borne flaviviruses and has important implications for studies that address the molecular basis of flavivirus pathogenesis. We plan to extend our strategy for vaccine development to the mosquito-borne West Nile flavivirus.

Major Areas of Research

- Molecular biology of positive strand RNA viruses
- Viral pathogenesis and immunity
- Vaccine development

Selected Recent Publications

Pletnev, A.G., Karganova, G.G., Dzhivanyan, T.I., Lashkevich, V.A., Bray, M. Chimeric Langat/Dengue viruses protect mice from heterologous challenge with the highly virulent strains of tick-borne encephalitis virus. *Virology* 274: 26-31, 2000.

Pletnev, A.G. Infectious cDNA clone of attenuated Langat tick-borne flavivirus (strain E5) and a 3′ deletion mutant constructed from it exhibit decreased neuroinvasiveness in immunodeficient (SCID) mice. *Virology* 282:288-300, 2001.

Laboratory of Intracellular Parasites

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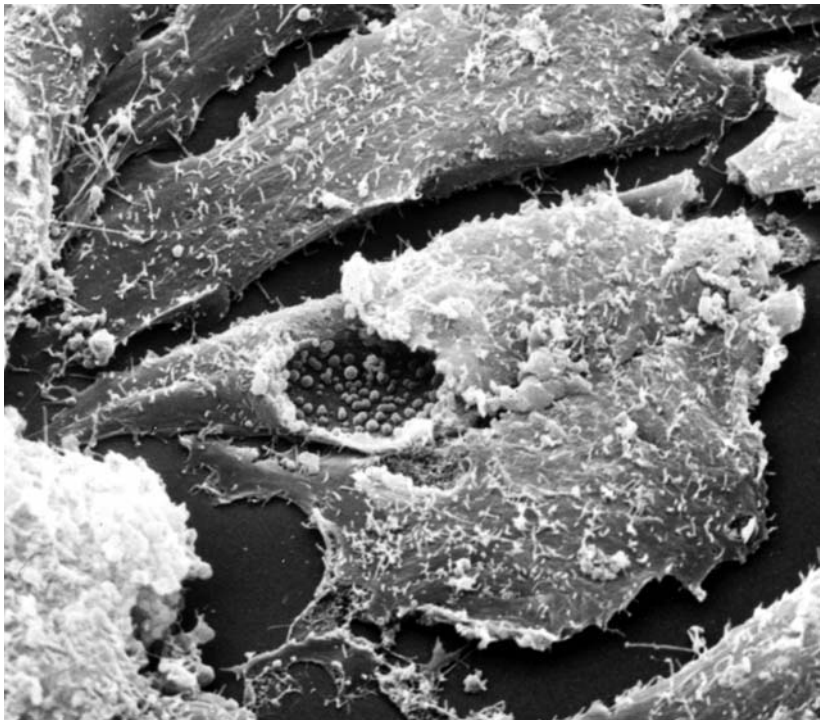
Host-Parasite Interactions Section
David W. Hackstadt, Ph.D., Head
Olivia Steele-Mortimer, Ph.D.



Research Activities

The Laboratory of Intracellular Parasites (LICP) investigates the biology, pathogenesis, and immunity of intracellular prokaryotic pathogens such as *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Coxiella burnetii*, *and Salmonella typhimurium.* The long-term goal of the laboratory is the development of new and effective control strategies against intracellular bacterial parasitic infections. Modern biological, molecular, and immunological tools are employed to understand parasite ligand-receptor interactions, parasite vesicle maturation and trafficking, parasite manipulation of host cell signal transduction pathways, parasite acquisition of nutrients, and host immune response to infection. Parasite and host gene expression are being analyzed by DNA microarray and real time PCR under experimental conditions that manifest both acute and persistent infection environments to profile novel parasite genes that function in the pathogenesis of infection. Animal models of infection are being employed to define immune effector mechanisms that function in adaptive protective immunity and for the testing of promising vaccine candidates.

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Dr. Caldwell obtained his doctorate from the University of Washington for the study of immunity to chlamydial infection. Following a postdoctoral position at the University of Washington, Dr. Caldwell joined the Francis I. Proctor Foundation and Department of Microbiology and Immunology at the University of California, San Francisco, as an assistant professor. There he continued his investigations on immunity to chlamydial infection. In 1980, Dr. Caldwell joined the NIAID Rocky Mountain Laboratory and in 1986 became Head of the Chlamydial Diseases Section. In 1990, Dr. Caldwell was appointed Chief of the Laboratory of Intracellular Parasites and in 1997 was elected to the National Institutes of Health (NIH) Senior Biomedical Research Service. Dr. Caldwell's research at NIH continues to focus on immunity to chlamydial infection. These studies have defined the molecular basis of cell-mediated protective immunity to chlamydial infection. Dr. Caldwell is the recipient of the NIH superior service award, NIH Directors Award, and NIH merit award.

Description of Research

Chlamydia trachomatis is an obligate intracellular prokaryote that primarily colonizes and infects the oculogenital mucosae of humans. These epithelia-tropic infections can cause trachoma, the worlds leading cause of preventable blindness, and sexually transmitted infections that can lead to pelvic inflammatory disease. An efficacious vaccine is the most promising approach to control chlamydial diseases, however little is known about immune mechanisms that function in adaptive immunity to infection, which has severely limited progress towards the development of an efficacious anti-chlamydial vaccine. The Immunology Section studies protective immunity against *Chlamydia trachomatis* mucosal infection of the murine female genital tract using *in vivo* depletion of lymphocyte subsets, adoptive transfer of lymphocyte clones, and infection of gene knock-out mice with known immunodeficiencies. Results from this work have identified a cooperative role for type 1 $CD4^+$ T cells and secretory IgA in mediating protective anti-chlamydial immunity. Ongoing studies are focused on understanding the cooperative effector functions of this immunity, identifying protective chlamydial antigens, and the design of rational immunization strategies for vaccination. A new discovery includes the identification of a chlamydial cytotoxin that could represent a novel virulence factor important in the pathogenesis of chlamydial diseases. Future research efforts will focus on describing the biological function of the chlamydial cytotoxin and exploring its potential as a target for protective neutralizing sIgA antibodies.

Major Areas of Research

- Immunity to chlamydial infection
- *Chlamydia* vaccine design

Selected Recent Publications

Shaw, J.H., Grund, V.R., Durling, L., Caldwell, H.D. Expression of genes encoding th1 cell-activating cytokines and lymphoid homing chemokines by chlamydia-pulsed dendritic cells correlates with protective immunizing efficacy. *Infect. Immun.* 69: 4667-72, 2001.

Wolf, K., Fischer, E., Mead, D., Zhong, G., Peeling, R., Whitmire, B., Caldwell, H.D. *Chlamydia pneumoniae* major outer membrane protein is a surface-exposed antigen that elicits antibodies primarily directed against conformation-dependent determinants. *Infect. Immun.* 69: 3082-91, 2001.

Morrison, S.G., Su, H., Caldwell, H.D., Morrison, R.P. Immunity to murine Chlamydia trachomatis genital tract reinfection involves B cells and CD4(+) T cells but not CD8(+) T cells. *Infect. Immun.* 68: 6979-87, 2000.

Su, H., Messer, R., Whitmire, W., Hughes, S., Caldwell, H.D. Subclinical chlamydial infection of the female mouse genital tract generates a potent protective immune response: implications for development of live attenuated chlamydial vaccine strains. *Infect. Immun.* 68: 192-6, 2000.

David W. (Ted) Hackstadt, Ph.D.

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Dr. Hackstadt received his doctorate from Washington State University studying herpes viruses. He performed postdoctoral work at the Rocky Mountain Laboratories (RML) where he studied physiological adaptations of the Q fever rickettsia, *Coxiella burnetii*, to intracellular parasitism. He subsequently was appointed Senior Staff Fellow and continued studies of *C. burnetii* as well as beginning work on *Chlamydia trachomatis.* Dr. Hackstadt left RML for an Associate Professorship in the Departments of Pathology and Microbiology at the University of Texas Medical School in Galveston. He returned to the LICP at RML in 1990 where he was appointed Head of the Host-Parasite Interactions Section. He serves on the editorial boards of the journals *Cellular Microbiology* and *Traffic* and is past president of the American Society for Rickettsiology.

Description of Research Program

The Host-Parasite Interactions Section studies the cellular and molecular biology of *Chlamydia*, *Rickettsia*, and other obligate intracellular parasites.

The vacuole in which chlamydiae replicate does not fuse with lysosomes, but the mechanisms of avoidance of lysosomal fusion are unknown. Researchers have found that the chlamydial inclusion interrupts an exocytic pathway, which delivers sphingolipids from the Golgi apparatus to the plasma membrane. Whereas the majority of intracellular parasites are thought to block maturation of the endocytic vesicle to a lysosome, chlamydiae dissociate themselves from this pathway and establish a functional interaction with an exocytic pathway. Collectively, the data suggest that the chlamydial inclusion occupies a site distal to the trans-Golgi apparatus, with properties of an exocytic vesicle in which fusion with the plasma membrane is inhibited or delayed. These results show potential not only for defining the interactions of chlamydiae with the host cell but for serving as a model system for other obligate intracellular pathogens that occupy vacuoles that do not fuse with lysosomes.

Modification of DNA structure by histone-like proteins appears to be a central regulatory mechanism governing the complex life cycle of *Chlamydia trachomatis.* Histone H1 homologs are expressed only during the late stages of the chlamydial life cycle concomitant with the reorganization of reticulate bodies to elementary bodies and play a major role in chromatin structure as well as in control of gene expression. Histone-mediated effects on chlamydial DNA topology are likely a component of a global regulatory scheme controlling differential gene expression in response to unidentified environmental conditions. The initial events in chlamydial differentiation, including the transition in properties of the endocytic vesicle to one that intersects an exocytic pathway, and dissociation of the condensed nucleoid complex remain significant challenges in understanding the pathogenic mechanisms of chlamydiae.

Major Areas of Research

- Chlamydia interactions with host cells
- Vesicle trafficking pathways

Selected Recent Publications

Scidmore, M.A., Hackstadt, T. Mammalian 14-3-3 beta associates with the *Chlamydia trachomatis* inclusion membrane via its interaction with IncG. *Mol. Microbiol.* 39:1638-50, 2001.

Wolf, K., Hackstadt, T. Sphingomyelin trafficking in *Chlamydia pneumoniae*-infected cells. *Cell. Microbiol.* 3:145-52, 2001.

Hackstadt T. Redirection of host vesicle trafficking pathways by intracellular parasites. *Traffic* 1:93-9, 2000.

Olivia Steele-Mortimer, Ph.D.

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Dr. Steele-Mortimer received her Ph.D. from the University of London for studies into the small GTP-binding protein rab5, a regulator of endocytic traffic in eukaryotic cells. This work was carried out at the European Molecular Biology Laboratory in Heidelberg, Germany. Dr. Steele-Mortimer then did postdoctoral research on interactions between the facultative intracellular pathogen *Salmonella typhimurium* and epithelial host cells at the University of British Columbia and at Washington University, St Louis. Her research group focuses on *S. typhimurium* infection of eukaryotic cells.

Description of Research Program

S. typhimurium is an economically important pathogen that causes gastroenteritis in humans. In order to cause disease the orally ingested bacteria must cross the intestinal epithelial barrier of the host. This process requires active invasion by the bacteria of nonphagocytic cells as well as the ability to survive and replicate intracellularly. Both of these processes are now known to be dependent on a number of bacterial effector proteins that are translocated into the host cell by a specialized Type III secretion system. The goals of our research are to characterize molecular mechanisms involved in intracellular survival of *S. typhimurium.* Several approaches, including microscopy, molecular biology and biochemistry are being used to identify host cell targets and pathways that are affected by the translocated effector proteins. One host cell signal transducer, which we have shown to be activated by *Salmonella*, is the pro-survival kinase Akt/protein kinase B. Activation is dependent on the translocated bacterial Type III effector protein SigD. Akt is recruited to *Salmonella*-induced membrane ruffles on the cell surface in the absence of SigD, however, phosphorylation and activation are dependent on the effector. Future work will focus on the mechanism of activation of Akt and on identification of other host cell factors involved in this process. Potentially these studies will provide valuable information on the mechanism of disease as well as on host cell regulatory pathways involved in cellular survival.

The regulation of membrane traffic in eukaryotic cells is an extremely complex and highly regulated process and consequentially the mechanisms involved have been difficult to elucidate. Intracellular *Salmonella* reside and replicate within a membrane bound vacuole known as the *Salmonella*-containing vacuole (SCV). This vacuole has limited interactions with the early endocytic pathway but lysosomal enzymes are not delivered, indicating that the bacteria prevent lysosome-endosome fusion. Further characterization of the SCV and on the role of bacterial effector proteins on SCV biogenesis should yield information on the mechanisms involved in host cell membrane traffic.

Major Areas of Research

- Membrane trafficking in infected host cells.
- Signal transduction events in *Salmonella*-infected cells.
- SCV (*Salmonella*-containing vacuole) biogenesis.

Selected Recent Publications

Steele-Mortimer, O., Knodler, L.A., Marcus, S.L., Scheid, M.P., Goh, B., Pfeifer, C.G., Duronio, V., Finlay, B.B. Activation of Akt/protein kinase B in epithelial cells by the *Salmonella typhimurium* effector sigD. *J. Biol. Chem.* 275:37718-24, 2000.

Steele-Mortimer, O., St-Louis, M., Olivier, M., and Finlay, B.B. Vacuole acidification is not required for survival of *Salmonella enterica* serovar *typhimurium* within cultured macrophages and epithelial cells. *Infect. Immun.* 68:5401-4, 2000.

Steele-Mortimer, O., Meresse, S., Gorvel, J.P., Toh, B.H. and Finlay, B.B. Biogenesis of *Salmonella typhimurium*-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway. *Cell. Microbiol.* 1:33-49, 1999.

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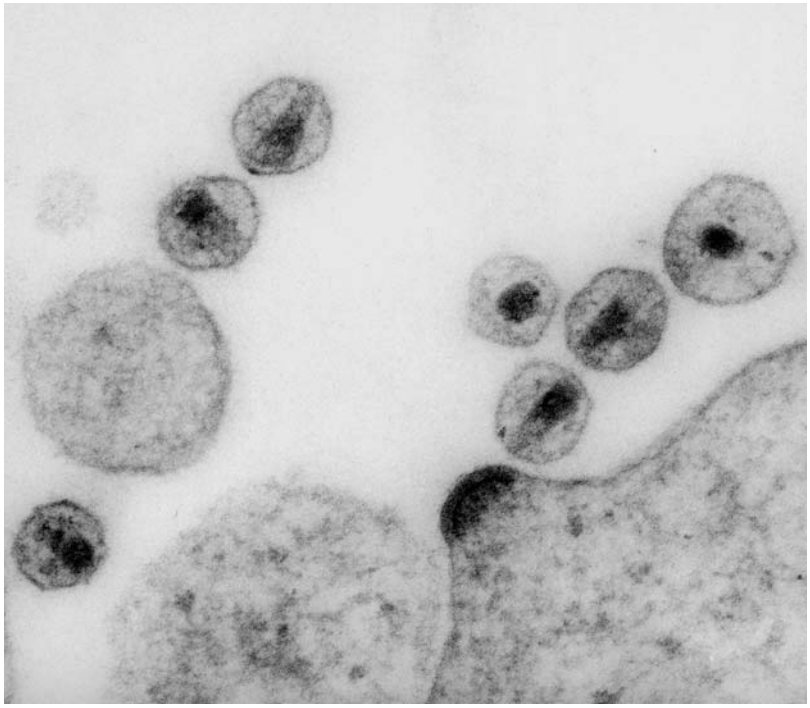
Vanessa M. Hirsch, D.V.M., D.Sc.



Research Activities

- Studies of the synthesis, metabolism, and immunogenicity of viral capsid and envelope proteins and their use to generate potentially useful antivirals or vaccines.
- Exploration of the structure and function relationship of retroviral accessory proteins synthesized during productive and chronic viral infections.
- Identification and characterization of cis-acting elements located within viral genomes that respond to regulatory factors.
- Development of animal models for investigations of viral pathogenesis, the identification of potentially useful antiviral agents, and the development of protective vaccines.
- Characterization of endogenous retroviral-related sequences present in mammalian genomes and their relationship to disease.
- Identification and properties of mycoplasmas associated with humans.

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Dr. Martin received an M.D. degree from Yale University School of Medicine in 1962, and following 2 years of clinical training in internal medicine at the University of Rochester, joined NIH as a Research Associate. He initially investigated the replication and gene regulation of SV40 and polyoma viruses and subsequently studied endogenous murine and human retroviral sequences. Since 1984, his research program has focused on HIV. Dr. Martin was appointed Chief of the Laboratory of Molecular Microbiology when it was established in 1981. He is a member of the National Academy of Sciences and recipient of numerous scientific awards.

Description of Research Program

In addition to ongoing investigations of HIV-1 gene regulation, viral structural protein synthesis and processing, and virion assembly, studies recently have been initiated to develop vaccine and disease models in subhuman primates. Thus far, the vaccine studies have examined whether the protective effects observed with attenuated SIV vaccines in macaque monkeys are applicable to the HIV-1 system. Toward this end, previously HIV-1-infected chimpanzees or SIV-HIV-1 (SHIV) chimeric virus-infected macaques are being "superchallenged" with heterologous viruses to ascertain the extent of resistance, if any, to a subsequent virus inoculation. Attempts to develop an HIV-1 relevant disease model have concentrated on the construction of novel SHIV viruses capable of causing CD4+ T cell loss and disease within a reasonable time frame (1 year or less). Recent work has concentrated on the very early host responses to primary virus infections including the reversibility of a declining clinical course. Vaccine studies have focused on identifying correlates of protection and the use of attenuated lentiviruses and live virus vectors in combination with protein boosts.

Major Areas of Research

- Studies of primate and murine retroviruses in cell culture and animal models
- HIV-1 vaccine studies
- Retroviral molecular biology

Selected Recent Publications

Cho, M.W., Kim, Y.B., Lee, M.K., Gupta, K.C., Ross, W., Plishka, R., Buckler-White, A., Igarashi, T., Theodore, T., Byrum, R., Kemp, C., Montefiori, D.C., Martin, M.A. Polyvalent envelope glycoprotein vaccine elicits a broader neutralizing antibody response but is unable to provide sterilizing protection against heterologous simian/human immunodeficiency virus infection in pigtailed macaques. *J. Virol.* 75: 2224-34, 2001.

Igarashi, T., Brown, C.R., Endo, Y., Buckler-White, A., Plishka, R., Bischofberger, N., Hirsch, V., Martin, M.A. Macrophage are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): implications for HIV-1 infections of humans. *Proc. Natl. Acad. Sci. USA* 98: 658-63, 2001.

Ogert, R.A., Lee, M.K., Ross, W., Buckler-White, A., Martin, M.A., Cho, M.W. N-linked glycosylation sites adjacent to and within the V1/V2 and the V3 loops of dual tropic human immunodeficiency virus type 1 isolate DH12 gp120 affect coreceptor usage and cellular tropism. *J. Virol.* 75: 5998-6006, 2001.

Cho, M.W., Lee, M.K., Chen, C.H., Matthews, T., Martin, M.A. Identification of gp120 regions targeted by a highly potent neutralizing antiserum elicited in a chimpanzee inoculated with a primary human immunodeficiency virus type 1 isolate. *J. Virol.* 74: 9749-54, 2000.

Endo, Y., Igarashi, T., Nishimura, Y., Buckler, C., Buckler-White, A., Plishka, R., Dimitrov, D.S., Martin, M.A. Short- and long-term clinical outcomes in rhesus monkeys inoculated with a highly pathogenic chimeric simian/human immunodeficiency virus. *J. Virol.* 74: 6935-45, 2000.

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Dr. Freed obtained his Ph.D. in cellular and molecular biology from the University of Wisconsin, Madison in 1990. He subsequently did postdoctoral work with Dr. Howard Temin at UW-Madison and with Dr. Malcolm Martin at the NIH. Dr. Freed became a tenure-track Investigator in 1997.

Description of Research Program

Our research program focuses on a variety of key aspects of HIV-1 assembly. Of particular interest are the role of viral and host factors in the targeting of Gag to the plasma membrane, the role of plasma membrane microdomains ("rafts") in membrane association and virus assembly, the mechanism by which the viral envelope glycoproteins are incorporated into virions, and the process by which budding particles pinch off from the surface of infected cells.

Major Areas of Research

- Envelope glycoprotein incorporation into HIV-1 virions
- Role of plasma membrane microdomains in HIV-1 replication
- Viral and cellular host factors in virus release

Selected Recent Publications

Kiernan, R.E., Ono, A., Englund, G, and Freed, E.O. Role of matrix in an early postentry step in the human immunodeficiency virus type 1 life cycle. *J. Virol.* 72: 4116-4126, 1998.

Freed, E.O. HIV-1 Gag proteins: diverse functions in the virus life cycle. *Virology* 251: 1-15, 1998.

Ono, A., and Freed, E.O. Binding of human immunodeficiency virus type 1 Gag to membrane: role of the matrix amino terminus. *J. Virol.* 73: 4136-4144, 1999.

Kiernan R.E., Ono, A., and Freed, E.O. Reversion of a human immunodeficiency virus type 1 matrix mutation affecting Gag membrane binding, endogenous reverse transcriptase activity, and virus infectivity. *J. Virol.* 73: 4728-4737, 1999.

Murakami, T., and Freed, E.O. The long cytoplasmic tail of gp41 is required in a cell type-dependent manner for HIV-1 envelope glycoprotein incorporation into virions. *Proc. Natl. Acad. Sci. USA* 97: 343-348, 2000.

Ono, A., Orenstein, J.M., and Freed, E.O. Role of the Gag matrix domain in targeting human immunodeficiency virus type 1 assembly. *J. Virol.* 74: 2855-2866, 2000.

Murakami, T., and Freed, E.O. Genetic evidence for an interaction between human immunodeficiency virus type 1 matrix and α-helix 2 of the gp41 cytoplasmic tail. *J. Virol.* 74: 3548-3554, 2000.

Ono, A., Demirov, D., and Freed, E.O. Relationship between human immunodeficiency virus type 1 Gag multimerization and membrane binding. *J. Virol.* 74: 5142-5150, 2000.

Vanessa M. Hirsch, D.V.M., Sc.D.

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Dr. Hirsch obtained her D.V.M. from the University of Saskatchewan in 1977 and became a diplomate of the American College of Veterinary Pathologists in 1984. She obtained a Doctor of Science degree at Harvard School of Public Health in 1988. Following 4 years as a research assistant professor at Georgetown University, she joined the Laboratory of Infectious Diseases in 1992 and then transferred to the Laboratory of Molecular Microbiology in 1998.

Description of Research Program

My group uses simian immunodeficiency virus infection of monkeys as a model to study the pathogenesis of human AIDS and to develop effective vaccine strategies. SIV is a highly relevant animal model for AIDS since it induces an immunodeficiency syndrome in macaque monkeys that is remarkably similar to that seen in HIV-infected humans. SIVs originate in primates of African origin including sooty mangabeys (SIVsm) and African green monkeys (SIVagm). My lab has been responsible for characterizing a number of these strains of SIV. Although the natural hosts for SIV are African primates, AIDS-like disease is observed only upon experimental inoculation of macaques, an Asian monkey species. My laboratory demonstrated that experimental infection of macaques with SIVagm results in AIDS, whereas infection of the natural host is asymptomatic. This discrepancy in the virulence of a common SIV strain for its natural host species and macaque monkeys provides a valuable model to study the underlying mechanisms of attenuation in the natural host species. The development of a vaccine for AIDS is the other focus of my research. My laboratory has focused on developing a vaccine based on the use of a highly attenuated vaccinia virus, modified vaccinia virus Ankara (MVA) to prime a cell-mediated immune response. MVA expressing SIV genes generated a robust cell mediated immune response, which resulted in significant modulation of viremia and improved survival following SIV challenge.

Major Areas of Research

- AIDS pathogenesis
- Evolution and origins of primate lentiviruses
- AIDS vaccine development

Selected Recent Publications

Beer, B.E., Bailes, E., Goeken, R., Dapolito, G., Coulibaly, C., Norley, S.G., Kurth, R., Gautier, J.-P., Gautier-Hion, A., Vallet, D., Sharp, P.M., and Hirsch, V.M. Simian immunodeficiency virus (SIV) from sun-tailed monkeys (*Cercopithecus solatus*): evidence for host-dependent evolution of SIV within the *C. lhoesti* superspecies. *J. Virol.* 73: 7734-7744, 1999.

Ourmanov, I., Brown, C.R., Moss, B., Carroll, M., Wyatt, L., Pletneva, L., Goldstein, S., Venzon, D., and Hirsch, V.M. Comparative efficacy of recombinant modified vaccinia virus Ankara expressing simian immunodeficiency virus (SIV) Gag-Pol and/or Env in macaques challenged with pathogenic SIV. *J. Virol.* 74: 2740-2751, 2000.

Goldstein, S., Ourmanov, I., Brown, C.R., Beer, B.E., Elkins, W.R., Plishka, R., Buckler-White, A., Hirsch, V.M. Wide range of viral load in healthy African green monkeys naturally infected with simian immunodeficiency virus. *J. Virol.* 74: 11744-53, 2000.

Goldstein, S., Brown, C.R., Dehghani, H., Lifson, J.D., Hirsch, V.M. Intrinsic susceptibility of rhesus macaque peripheral CD4(+) T cells to simian immunodeficiency virus *in vitro* is predictive of *in vivo* viral replication. *J. Virol.* 74: 9388-9395, 2000.

Kuan-Teh Jeang, M.D., Ph.D.

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Dr. Jeang obtained his M.D. and Ph.D. degrees from the Johns Hopkins University School of Medicine for the study of the molecular biology of human herpesviruses. Following postdoctoral research at the National Cancer Institute, he joined the Laboratory of Molecular Microbiology in 1988 and became the Head of the Molecular Virology Section in 1993. His research focuses on the transcriptional regulation of HIV-1 and the transforming properties of HTLV-1. Dr. Jeang serves on several editorial boards including those for the *Journal of Biological Chemistry* and *the Journal of Virology.*

Description of Research Program

Our research program focuses on four areas: molecular regulation of HIV-1, molecular regulation of HTLV-1, cell cycle checkpoints, and development of HIV-1-specific small molecule inhibitors.

Major Areas of Research

- HIV-1 transcription
- HTLV-1 transcription and transformation
- Cell cycle checkpoints
- Molecular antivirals

Selected Recent Publications

Jeang, K.-T., Xiao, H. and Rich, E.A. Multifaceted activities of the HIV-1 transactivator of transcription Tat. *J. Biol. Chem.* 274: 28837-40, 1999.

Kiernan, R.E., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K-T., Benkirane, M. and Van Lint, C. HIV-1 Tat transcriptional activity is regulated by acetylation. *EMBO J.* 18: 6106-6118, 1999.

Giordano,V., Jin, D.-Y., Rekosh, D., and Jeang, K.-T. Intravirion targeting of a functional anti-HIV ribozyme directed to *pol. Virology* 267: 174-184, 2000.

Jin, D.-Y., Wang, H.-L., Zhou, Y., Chun, A., Kibler, K., Hou, Y.-D., Kung, H.-F. and Jeang, K-T. Hepatitis C virus core protein-induced loss of LZIP function correlates with cellular transformation. *EMBO J.* 19: 729-740, 2000.

Xiao, H., Neuveut, C., Tiffany, H. L., Benkirane, M., Rich, E. A., Murphy, P. M., and Jeang, K.-T. Selective CXCR4-antagonism by Tat: implications for in vivo expansion of co-receptor use by HIV-1. *Proc. Natl. Acad. Sci. USA* 97: 11466-11471, 2000.

Van, P. L., Yim, K.-W., Jin, D.-Y., Dapolito, G., Kurimasa, A., and Jeang, K.-T. Genetic evidence of a role for ATM in functional interaction between human T-cell leukemia virus type 1 Tax and p53. *J. Virol.* 75: 396-407, 2001.

Kibler, K.V., and Jeang, K.-T. CREB/ATF-dependent repression of cyclin A by the human T-cell leukemia virus type-I Tax protein. *J. Virol.* 75: 2161-2173, 2001.

Jeang, K.-T. Functional activities of the human T-cell leukemia virus type I Tax oncoprotein: cellular signaling through NFκB. *Cytokine & Growth Factor Reviews* 12: 207-217, 2001.

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Dr. Kozak obtained her Ph.D. from Yale University for the development of the first panel of Chinese hamster X mouse somatic cell hybrids for mouse genetic analysis. She conducted postdoctoral research in the Laboratory of Viral Diseases and later joined the Laboratory of Molecular Microbiology, becoming Section Head in 1992. Her research centers on the genetic basis of resistance to murine leukemia viruses and virus-induced disease and the development of the mouse genetic linkage map. Dr. Kozak serves as Associate Editor for four journals and is Chair of the Mouse Chromosome 5 Committee.

Description of Research Program

My laboratory has a longstanding interest in the identification and characterization of genes that affect susceptibility to mouse leukemia viruses (MLVs) and to the diseases these viruses induce. Inbred strains of laboratory mice and wild mouse species differ in their virus susceptibility, and numerous genes have been identified that are responsible for these differences. We are currently engaged in efforts to characterize these mouse genes and determine the mechanisms responsible for resistance. Among the genes currently under study are the following:

The *Fv1* gene is responsible for relative resistance to some MLV subgroups. The target of Fv1 resistance is the viral gene encoding the virion capsid protein. We are in the process of analyzing mutated viruses carrying specific amino acid substitutions and deletions to describe the full range of Fv1 restricted phenotypes.

Rmcf is responsible for resistance to the leukemogenic polytropic host range group of MLVs. We have identified a novel copy of the viral envelope gene integrated into the mouse genome at or near the *Rmcf* locus. Studies are in progress to determine if this gene functions to block infection by exogenous virus.

Most mouse strains contain a serum factor capable of inactivating the xenotropic subgroup of MLVs. We have determined that production of this factor is under single gene control and have determined its location on the mouse genetic map. We are in the process of evaluating candidate genes in the region.

In addition to these studies, we have shown that many wild mouse species, like laboratory mice, contain chromosomally integrated viral genes. We have now determined that many of these wild mouse proviruses can contribute to the production of infectious virus. We are currently engaged in characterizing the replication properties and disease potential of these novel isolates.

Major Areas of Research

- Genetics of resistance to mouse retroviruses
- Naturally occurring mouse retroviruses
- Mouse genomics

Selected Recent Publications

Jung, Y.T., and Kozak, C.A. A single amino acid change in the murine leukemia virus capsid gene responsible for the Fv1nr phenotype. *J. Virol.* 74: 5385-5387, 2000.

Rajan, L., Broussard, D., Lozano, M., Lee, C.G., Kozak, C.A., and Dudley, J.P. The *c-myc* locus is a common integration site in type B retrovirus-induced T-cell lymphomas. *J. Virol.* 74: 2466-2471, 2000.

Rovescalli, A.C., Cinquanta, M., Ferrante, J., Kozak, C.A., and Nirenberg, M. The mouse NKX-1.2 homeobox gene: alternative RNA splicing at canonical and noncanonical splice sites. *Proc. Natl. Acad. Sci. USA* 97: 1982-1987, 2000.

Tailor, C.S., Nouri, A., Lee, C.G., Kozak, C., and Kabat, D. Cloning and characterization of a cell surface receptor for xenotropic and polytropic murine leukemia viruses. *Proc. Natl. Acad. Sci. USA* 96: 927-932, 1999.

Jonathan Silver, M.D.

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Dr. Silver received an M.S. in physics from Stanford University and an M.D. from Harvard University. He came to NIH in 1980 to work on murine retroviruses and became a tenured Senior Investigator in 1987. His laboratory has studied host genes affecting susceptibility to retroviruses and pioneered applications of PCR to retrovirology such as inverse PCR and sensitive detection of reverse transcriptase. His group is currently using alphavirus vectors to study processes mediated by retroviral structural proteins, such as virus entry and particle formation. It is also developing technology for performing multiple PCRs in parallel in an array format for applications in genetics and infectious diseases.

Description of Research Program

We are interested in the membrane fusion processes that must occur when enveloped viruses enter and exit cells, and how this relates to membrane inhomogeneity in eukaryotic cells in general. Using murine retroviruses (MLV) and Sindbis virus as model systems, we recently found that the receptor protein for the ecotropic class of MLV is located in specialized portions of the cell membrane termed "rafts", and that cholesterol depletion, which disrupts rafts, inhibits fusion mediated by MLV envelope as well as Sindbis envelope. We want to understand what determines raft localization of the MLV receptor, and how the specialized lipid environment of rafts might affect fusion and budding. In addition to usual techniques of molecular biology, we make extensive use of confocal microscopy to image viral proteins and cell receptors in living cells. Where possible, we seek to understand membrane fusion in physical as well as biological terms.

Major Areas of Research

- Retrovirology
- Membrane fusion

Selected Recent Publications

Silver, J., Mi, Z., Takamoto, K., Bungay, P. and Powell, A. Controlled formation of low-volume liquid pillars between plates with a lattice of wetting patches by use of a second immiscible fluid. *J. Colloid Interface Sci.* 219:81-89, 1999.

Kazachkov, Y., Long, D., Wang, C., and Silver, J. Changes in a murine leukemia virus (MLV) receptor encoded by an alphavirus vector during passage in cells expressing the MLV envelope. *Virology* 267; 124-132, 2000.

Lu, X. and Silver, J. Ecotropic murine leukemia virus receptor is physically associated with caveolin and membrane rafts. *Virology* 276: 251-258, 2000.

Lu, X. and Silver, J. Transmission of replication-defective Sindbis helper vectors encoding capsid and envelope proteins. *J. Virological Methods* 91: 59-65, 2001.

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Dr. Strebel obtained his Ph.D. in 1985 from the University of Heidelberg in Germany for the functional characterization of picornaviral proteins. Following postdoctoral research at the University of Heidelberg, he joined the Laboratory of Molecular Microbiology in 1986 and received tenure in 1997. His research focuses on the biological and biochemical characterization of HIV proteins and the role of host factors in the establishment of viral infection. Dr. Strebel is *ad hoc* reviewer for numerous scientific journals.

Description of Research Program

HIV encodes a number of accessory genes not commonly found in other retroviruses. A major interest of laboratory researchers is the investigation of the biological and biochemical functions of the HIV accessory proteins Vif and Vpu in an attempt to understand their precise role in virus replication. The goal is to characterize cellular factors involved in Vif or Vpu function. From the laboratory's studies on Vpu, insights are expected into general principles of protein degradation from the endoplasmic reticulum and into mechanisms involving late stages of virus production, in particular the involvement of ion channels in the secretory pathway. From studies on Vif, staff members not only expect to gain insights into the function of this viral factor but also expect to learn more about the role of the cytoskeleton in virus replication. Finally, it is hoped that the studies will provide a basis for the assessment of viral proteins as potential antiviral targets.

The Vpu gene is unique to HIV-1 and encodes a small membrane protein. Vpu regulates virus release from the cell surface and degradation of CD4 in the endoplasmic reticulum. These two biological activities of Vpu are based on two independent and distinct molecular mechanisms that can be attributed to separable structural domains of Vpu. The laboratory established that Vpu-mediated CD4 degradation involves the ubiquitin-dependent proteasome pathway and requires an interaction with a novel cellular protein, h-b TrCP. H-b TrCP was found to engage in ternary complexes with Vpu and CD4—requiring phosphorylation of Vpu, and represents a direct link to Skp1p, a known component of E3 ubiquitin ligase complexes.

Vif is a 23-kDa basic protein, which has an important function in regulating infectivity of progeny virions. Laboratory researchers analyzed the role of Vif by studying its subcellular distribution by cell fractionation as well as confocal microscopy. It was found that a substantial portion of intracellular Vif protein is associated with the cytoskeleton, specifically intermediate filaments. The association of Vif with intermediate filaments is specific and can result in the reorganization of the cytoskeletal network. More recently, laboratory researchers investigated the packaging of Vif into virions and found that Vif is associated with the viral nucleoprotein complex and may thus play a role in virus assembly and/or maturation.

Major Areas of Research

- Biological and biochemical functions of HIV accessory proteins
- Characterization of cellular factors involved in Vif and Vpu function

Selected Recent Publications

Khan, M.A., Aberham, C., Kao, S., Akari, H., Gorelick, R., Bour, S., Strebel, K. HIV-1 Vif protein is packaged into the nucleoprotein complex through an interaction with viral genomic RNA. *J. Virol.* 75:7252-65, 2001.

Bour, S., Perrin, C., Akari, H., Strebel, K. The human immunodeficiency virus type 1 Vpu protein inhibits NF-kappa B activation by interfering with beta TrCP-mediated degradation of I kappa B. *J. Biol. Chem.* 276:15920-8, 2001.

Akari, H., Arold, S., Fukumori, T., Okazaki, T., Strebel, K., Adachi, A. Nef-induced major histocompatibility complex class I down-regulation is functionally dissociated from its virion incorporation, enhancement of viral infectivity, and CD4 down-regulation. *J. Virol.* 74:2907-12, 2000.

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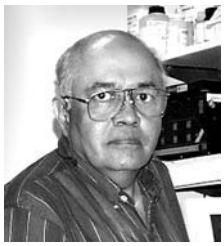

Dr. Venkatesan is a board certified anatomic and clinical pathologist (1977) who obtained his postgraduate training in pathology and biochemistry at the University of Pittsburgh Health Center. Following his pathology residency at the University of Pittsburgh Health Center Hospitals and postdoctoral research in mRNA processing and nucleic acid enzymology at the University of Pittsburgh, Dr. Venkatesan joined NIAID. He has been engaged in molecular virologic studies of human immunodeficiency viruses and AIDS pathogenesis and shifted priorities in 1999 to focus on the biological regulatory mechanisms of chemokine receptors and related G-protein coupled receptors. He is the author of nearly seventy publications, serves a reviewer for major biomedical journals, and is an elected member of the American Society of Biological Chemists.

Description of Research Program

One interest of this laboratory has been the mechanisms of eukaryotic mRNA processing and transport. Scientists study the HIV-1 Rev protein both as a model system and to understand the different operational circuits in the HIV life cycle. Rev protein of HIV regulates the temporal switch from the early regulatory to the late lytic phase of the virus life cycle. The Rev regulatory protein of HIV is a basic nuclear protein that enables the transport of the viral RNAs by binding to a highly structured RRE (Rev responsive element) RNA. Studies of Rev and RRE RNA were targeted to analyze the various functional motifs of the Rev protein and to identify and characterize the function of the putative cellular factors that may bind RRE RNA, Rev, or both. Among the latter was a cellular protein that bound to RRE RNA and was a potent competitive inhibitor of double-stranded RNA activation of interferon-induced PKR kinase. Researchers have also conducted biophysical studies on the nature of Rev binding to RRE with the goal of developing specific inhibitors by rational design.

The other major focus of this laboratory in recent years has been the structure-function correlation of human chemokine receptors and biochemical mechanisms of G-protein coupled receptor signaling. In particular, the structural requirements and mechanisms relating to the biological function and HIV usage by the CC and CXC chemokine receptors, CCR5 and CXCR4, are being studied. The ongoing studies also address the mechanistic differences underlying ligand-mediated internalization and desensitization between the CC and CXC chemokine receptors. Other studies examine other T-cell receptors that may be linked to chemokine receptors and may thus be co-modulated during chemokine signaling. Modulation of cell surface expression of CCR5 and CXCR4 in various cell types during acute infection with HIV and during natural disease is investigated in terms of HIV evolution and tropism switch.

Major Areas of Research

- HIV gene regulation, emphasizing RNA binding proteins, immune modulation and receptor signaling
- Pharmacological aspects of chemokine receptor signaling

Selected Recent Publications

Jeong, K.S., Nam, Y.S. and Venkatesan, S. Deletions near the N-terminus of HIV-1 Rev reduce RNA binding affinity and dominantly interfere with Rev function irrespective of the RNA target. *Arch. Virol.* 145: 2443-2467, 2000.

Nam, Y.S., Petrovic, A., Jeong, K.S., and Venkatesan, S. Exchange of the basic domain of HIV-1 Rev for a poly-arginine stretch expands the RNA binding specificity and a minimal arginine cluster is required for optimal RRE RNA binding affinity, nuclear accumulation, and *trans*-activation. *J. Virol.* 75: 2957-2971, 2001.

Venkatesan, S., Petrovic, A., Kim, Y.O., Weissman, D. and P.M. Murphy. A membrane proximal basic domain and cysteine cluster in the C-terminal tail of CCR5 constitute a bi-partite motif critical for cell surface expression. *J. Biol. Chem.* 276: 40133-45, 2001.

Laboratory of Parasitic Diseases

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Robert Gwadz, Ph.D., Assistant Chief

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Thomas Nutman, M.D., Head

Intracellular Parasite Biology Section
David Sacks, Ph.D., Head

Biochemical and Biophysical Parasitology Section
James Dvorak, Ph.D., Head

Gastrointestinal Parasites Section
Theodore Nash, M.D., Head

Cell Biology Section
Dennis M. Dwyer, Ph.D., Head

Malaria Cell Biology Section
Louis Miller, M.D., Head
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Malaria Genetics Section
Thomas Wellems, M.D., Ph.D., Head
Xin-zhuan Su, Ph.D., Unit Head

Opportunistic Parasitic Diseases Section
Franklin Neva, M.D., Head

Immunobiology Section
Alan Sher, Ph.D., Head
Thomas Wynn, Ph.D., Unit Head

Research Activities

The Laboratory of Parasitic Diseases (LPD) conducts basic and applied research on the prevention, control, and treatment of a variety of parasitic diseases. Biochemical and molecular studies are used to identify the stages and manner in which functional immune responses occur, the structure of functional proteins, the response of parasites to drugs, and the pathogenesis of parasitic diseases. Investigations of factors that influence vector capacity, including genetic determinants and the molecular components of anopheline mosquitoes, are areas of special interest. A new area of focus will be insect physiology of feeding and its influence on vector capacity. The LPD also includes a clinical group that conducts studies of selected patients at the NIH Clinical Center or at collaborating institutions in other countries. Specific projects include: elucidation of interactions of intracellular parasites, such as *Leishmania*, *Toxoplasma*, and *Trypanosoma cruzi*, with host cells; identification of functionally important proteins of protozoan and helminth parasites; identification of membrane enzymes and transport proteins of *Leishmania* and developmentally regulated molecules related to their virulence and differentiation; immunologic and molecular factors affecting pathogenicity of *Strongyloides; Plasmodium* genomic structure and inherited determinants that affect drug responses, pathogenic mechanisms, and infectivity to the host and vector in malaria; and, cooperative research in a Malaria Research and Training Center (MRTC) in Bamako, Mali (West Africa), for studies on pathogenesis, malarial drug resistance, and the population biology of anopheline mosquitoes as a prelude to malaria control schemes based on the replacement of vector populations with mosquitoes unable to transmit the malaria parasite. In cooperation with Malian staff, the MRTC is being developed as a site for testing malaria vaccine candidates.

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A.B.–Oberlin College; Ph.D.–Cell Biology, University of California, San Diego; Postdoctoral Fellow–National Institute for Medical Research, London, U.K.; Research Associate–Peter Bent Brigham Hospital, Boston, MA; Assistant Professor–Department of Pathology, Harvard Medical School and Brigham and Woman's Hospital, Boston, MA.

Description of Research Program

The major focus of the Immunobiology Section of the Laboratory of Parasitic Diseases is the study of host resistance and immune regulation in parasitic and other infections of global importance. The ultimate goal of this work is immunologic disease intervention in the form of vaccination or immunotherapy. At the same time, our research on the host response to infection has provided insights into the effector functions and regulatory mechanisms utilized by the vertebrate immune system. Much of the work of the Section is focused on the immunologic analysis in murine models of diseases induced by parasitic and bacterial agents (e.g. *Schistosoma mansoni*, *Toxoplasma gondii*, *Mycobacterium* spp., *Helicobacter* spp.). Current activities include functional mapping of host-resistance pathways, studies on IL-10/IL-12 regulation of immunopathology, analysis of Th1 and Th2 differentiation in parasitic infections which induce polarized lymphokine responses, and the investigation of dendritic cell function in the initiation of microbial immunity. A separate AIDS program examines mechanisms by which co-infection with intracellular pathogens may promote HIV-1 disease expression and focuses on antigen-presenting cells as reservoirs for induction of latent virus.

Major Areas of Research

- Initiation and regulation of T-lymphocyte responses to intracellular parasitic and bacterial pathogens
- Effector mechanisms of host resistance to parasitic and bacterial infections
- Role of IL-12 in host resistance and disease
- Innate recognition of pathogens by dendritic cells
- Role of IL-10 in regulation of infection-induced immunopathology

Selected Recent Publications

Yap, G.S. and Sher A. Effector cells of both nonhemopoietic and hemopoietic origin are required for interferon (IFN)-gamma- and tumor necrosis factor (TNF)-alpha-dependent host resistance to the intracellular pathogen, *Toxoplasma gondii. J. Exp. Med.* 189: 1083-1092, 1999.

Reis e Sousa, C., Yap, G., Schulz, O., Rogers, N., Schito, M., Aliberti, J., Hieny, S., and Sher, A. Paralysis of dendritic cell IL-12 production by microbial products prevents infection-induced immunopathology. *Immunity* 11: 637-47, 1999.

Aliberti, J., Reis e Sousa, C., Schito, M., Hieny, S., Wells, T., Huffnagle, G.B. and Sher, A. CCR5 provides a signal for microbial induced production of IL-12 by CD8+ dendritic cells. *Nature Immunol.* 1: 83-7, 2000.

Yap, G., Pesin, M., and Sher, A. Cutting edge: IL-12 is required for the maintenance of IFN-gamma production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii. J. Immunol.* 165: 628-31, 2000.

Thomas E. Wellems, M.D., Ph.D.

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Dr. Wellems obtained his M.D. and Ph.D. degrees from the University of Chicago. The subject of his Ph.D. studies was the molecular pathogenesis of the sickle hemoglobin mutation. Following residency in internal medicine at the Hospitals of the University of Pennsylvania, he joined the NIAID Laboratory of Parasitic Diseases in 1984 and became Section Head in 1991. Research in Dr. Wellems' Section focuses on genetic determinants of drug response and pathogenesis in *Plasmodium falciparum* malaria. He is a recipient of the NIH Director's Award and has been elected to the Senior Biomedical Research Staff.

Description of Research Program

The Malaria Genetics Section of the Laboratory of Parasitic Diseases conducts basic research on factors that govern the drug response, pathogenesis, and transmission of malaria. The work incorporates strategies of linkage mapping, field population surveys, gene manipulation, and gene product analysis, with a view to the discovery of fundamental biological information that will be of use in the development of new diagnostics, therapeutics, and control measures against the disease.

Current projects include investigations of molecular mechanisms of drug resistance, particularly the resistance of malaria strains to such crucial antiparasite drugs as chloroquine, quinine, and mefloquine; gene transcription switches and DNA recombination events responsible for the antigenic variation and immune evasion of parasitized red blood cells; epidemiology of hemoglobins C and S (sickle-cell) and their protection against severe malaria in African children; and a genetic defect of chromosome 12 that adversely affects the development of male gametocytes.

Major Areas of Research

- Mechanisms of parasite resistance to antimalarial drugs
- Pathogenesis and transmission of *Plasmodium falciparum* malaria
- Immune evasion of parasitized red blood cells

Selected Recent Publications

Nomura, T., Carlton, J.M., Baird, J.K., del Portillo, H.A., Fryauff, D.J., Rathore, D., Fidock, D.A., Su, X., Collins, W.E., McCutchan, T.F., Wootton, J.C., Wellems, T.E. Evidence for different mechanisms of chloroquine resistance in 2 *Plasmodium* species that cause human malaria. *J. Infect. Dis.* 183:1653-61, 2001.

Djimde, A., Doumbo, O.K., Cortese, J.F., Kayentao, K., Doumbo, S., Diourte, Y., Dicko, A., Su, X.Z., Nomura, T., Fidock, D.A., Wellems, T.E., Plowe, C.V., Coulibaly, D. A molecular marker for chloroquine-resistant falciparum malaria. *N. Engl. J. Med.* 344:257-63, 2001.

Deitsch, K., Driskill, C., Wellems, T. Transformation of malaria parasites by the spontaneous uptake and expression of DNA from human erythrocytes. *Nucleic Acids Res.* 29:850-3, 2001.

Fidock, D.A., Nomura, T., Talley, A.K., Cooper, R.A., Dzekunov, S.M., Ferdig, M.T., Ursos, L.M., Sidhu, A.B., Naude, B., Deitsch, K.W., Su, X.Z., Wootton, J.C., Roepe, P.D., Wellems, T.E. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol. Cell.* 6:861-71, 2000.

Freitas-Junior, L.H., Bottius, E., Pirrit, L.A., Deitsch, K.W., Scheidig, C., Guinet, F., Nehrbass, U., Wellems, T.E., Scherf, A. Frequent ectopic recombination of virulence factor genes in telomeric chromosome clusters of *P. falciparum*. *Nature* 407:1018-22, 2000.

Su, X., Ferdig, M.T., Huang, Y., Huynh, C.Q., Liu, A., You, J., Wootton, J.C., Wellems, T.E. A genetic map and recombination parameters of the human malaria parasite *Plasmodium falciparum*. *Science* 286:1351-3, 1999.

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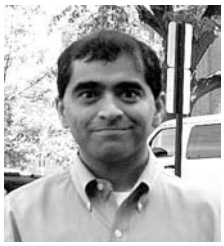

Awards: ICAAC Young Investigator Award (Am. Soc. of Microbiology); Young Investigator Award (Am. Soc. of Tropical Medicine & Hygiene).

Memberships: American Society of Tropical Medicine and Hygiene; Biophysical Society.

Description of Research Program

We study membrane transport events in the human malaria parasite, *Plasmodium falciparum*, with the goal of understanding basic physiological processes such as parasite nutrient acquisition, signal transduction, motility, and volume regulation. We have focused on the adaptations of the parasite that allow survival inside the red blood cell (RBC), one of the most nutrient-poor and metabolically inert cells of our bodies.

We used the on-cell and whole-cell patch-clamp methods to study how the parasite changes its host RBC membrane. These methods revealed an unusual ion channel, the plasmodial surface anion channel (PSAC). PSAC provides a mechanistic explanation for the long-known increased permeability of infected RBCs to nearly all nutrient solutes.

We also identified a separate channel on the parasitophorous vacuolar membrane, which surrounds the intraerythrocytic parasite. This channel is 50 times more conductive than PSAC, passes all charged and uncharged solutes smaller than 1400 dal, and functions as a molecular sieve for nutrients in RBC cytosol.

The identification and characterization of these unusual channels allowed us to propose a sequential diffusive pathway for nutrient acquisition by the intraerythrocytic parasite. In our model, essential solutes such as sugars, amino acids, purines (all present in high concentrations in serum) enter the RBC cytosol via PSAC, cross the vacuolar membrane through the large non-selective channel, and are finally acquired by the parasite via specific carriers on the parasite's plasma membrane.

Major Areas of Research

- Transport physiology of the malaria parasite
- Molecular biology and informatics in malaria
- Design and testing of channel blockers for new antimalarial therapies

Selected Recent Publications

Desai, S.A., Bezrukov, S., and Zimmerberg, J. A voltage-dependent channel involved in nutrient uptake by malaria parasite-infected red blood cells. *Nature* 406:1001-1005, 2000.

Desai, S.A. and Rosenberg, R.L. Pore size of the malaria parasite's nutrient channel. *Proc. Natl. Acad. Sci. USA* 94: 2045-2049, 1997.

Desai, S.A., McCleskey, E.W., Schlesinger, P.H., and Krogstad, D.J. A novel pathway for Ca^{++} entry into *Plasmodium falciparum*-infected blood cells. *Am. J. Trop. Med. & Hyg.* 54:464-470, 1996.

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Dr. Dvorak earned a Ph.D. from the University of Pennsylvania in biophysics/molecular biology research in 1968. His interests include: 1) obligate intracellular protozoan parasitism, 2) intraspecific genetic heterogeneity of parasitic protozoa, 3) development and utilization of biophysical methods for biomedical research, and 4) computer-assisted simulation and modeling of biological systems. He is the recipient of the Japan Society for the Promotion of Science Distinguished Scientist Award and the NIH Directors Award.

Description of Research Program

The Biochemical and Biophysical Parasitology program is involved in three major research areas: 1) the development of atomic force microscopy (AFM) for studies of biomedical problems, in general, and, more specifically, obligate intracellular protozoan parasites; 2) elucidation of the mechanism(s) involved in the high level of intra-specific diversity of medically important parasitic protozoa; 3) elucidation of the mechanism(s) involved in the process of interiorization by the obligate intracellular phase of medically-important parasitic protozoa.

The AFM project represents a new initiative in the LPD. Major emphasis initially was directed towards the development of preparative protocols and the interpretation of both qualitative and quantitative AFM data. We developed both qualitative and quantitative methods for *in vitro* studies of vertebrate cells by AFM and applied these methods to studies of macro-molecular events occurring during the life cycle of medically important parasitic protozoa as well as studies of changes in physical properties during the mitotic cycle of vertebrate cells under physiological conditions. Current research is directed towards developing the AFM as a tool to estimate thermodynamic parameters of complex, biologically important molecules.

The program was responsible for the original observation that a high level of intra-specific diversity exists in *Trypanosoma cruzi*, the causative agent of Chagas' disease in man. We have shown that intra-specific diversity extends from the DNA level to the presentation and course of disease in experimental animals. We have also observed that diversity can arise spontaneously in the laboratory. The mechanism for this phenomenon is unknown. We are, at present, attempting to develop methods to induce high levels of intra-specific diversity in *T. cruzi.*

Major Areas of Research

- AFM studies of obligate intracellular protozoan parasites
- Mechanism(s) involved in the high level of intra-specific diversity of medically important parasitic protozoa
- Mechanism(s) involved in interiorization by obligate intracellular phase of parasitic protozoa

Selected Recent Publications

Zieler, H. and Dvorak, J.A. Invasion *in vitro* of mosquito midgut cell by the malaria parasite proceeds by a conserved mechanism and results in death of the invaded midgut cells. *Proc. Natl. Acad. Sci. USA* 97:11516-21, 2000.

Dvorak, J.A., Kobayashi, S., Abe, K., Fujiwara, T., Takeuchi, T., and Nagao, E. The application of the atomic force microscope to studies of medically important protozoan parasites. *Journal of Electron Microscopy* 49: 429-435, 2000.

Nagao, E., Nishijima, H., Akita, S., Nakayama, Y., and Dvorak, J.A. Cell biological application of carbon nanotube probes for atomic force microscopy: comparative studies of malaria-infected erythrocytes. *Journal of Electron Microscopy* 49: 453-458, 2000.

Nagao, E., Kaneko, O., Dvorak, J.A. *Plasmodium falciparum*-infected erythrocytes: qualitative and quantitative analyses of parasite-induced knobs by atomic force microscopy. *Journal of Structural Biology* 130: 34-44, 2000.

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Dr. Dwyer received his Ph.D. from the University of Massachusetts at Amherst for studies concerning the biochemistry and cell biology of several pathogenic protozoa. Following 2 years of postdoctoral research at the Rockefeller University and 4 years as an Assistant Professor at that institution, he joined the Laboratory of Parasitic Diseases in 1976 as a Senior Investigator. Concurrently, he has been an Adjunct Professor on the graduate faculties of The Rockefeller University, the Cornell Medical College and his *alma mater*. His research group focuses on the basic cell and molecular biology of *Leishmania*, an important protozoan pathogen of humans worldwide. He serves on several journal editorial boards and various national and international grant review panels.

Description of Research Program

The cell and molecular biology of the protozoan pathogen, *Leishmania*, are investigated as a model of human parasitism. The goals of this research are to identify and characterize the basic mechanisms which facilitate this parasite's survival in its human and insect vector hosts. To that end, the basic biochemical functions and the structure of the genes encoding surface membrane and secreted proteins (enzymes) are investigated to define their roles in parasite survival and development. Several unique parasite enzymes are currently being studied: a family of constitutively expressed acid phosphatases, a bi-functional surface membrane 3´-nucleotidase/nuclease and a developmentally expressed chitinase. Homologous gene-deletion, over-expression and anti-sense methods are being used to determine/verify whether these proteins are critical to the survival of these organisms. In addition, chimeric proteins containing epitope tags (e.g. His-6, green fluorescent protein, etc.) are being over-expressed in these cells to study the cellular trafficking and targeting of these unique parasite enzymes. Characterization of such essential parasite proteins should provide valuable new targets toward the development of improved diagnostics and innovative chemo- and immunotherapeutic interventions for this important group of human pathogens.

Major Areas of Research

- Cell biology: secretory and endocytic trafficking
- Biochemistry of surface membrane and secreted enzymes
- Molecular biology: gene structure/function and expression

Selected Recent Publications

Debrabant, A., Ghedin, E. and Dwyer, D.M. Dissection of the functional domains of the *Leishmania donovani* surface membrane 3'-nucleotidase/nuclease, a unique member of the class-I nuclease family. *J. Biol. Chem.* 275: 16366-16372, 2000.

Shakarian, A.M. and Dwyer, D.M. Pathogenic *Leishmania* secrete antigenically related chitinases, which are encoded by a highly conserved gene locus. *Exp. Parasitol.* 94: 238-242, 2000.

Yamage, Y., Debrabant, A. and Dwyer, D.M. Molecular characterization of a hyperinducible, surface membrane-anchored, class-I nuclease of a trypanosomatid parasite. *J. Biol. Chem.* 275: 36369-36379, 2000.

Ghedin, E., Debrabant, A., Engel, J.C. and Dwyer, D.M. Secretory and endocytic pathways converge in a dynamic endosomal system in a primitive protozoan. *Traffic* 2: 175-188, 2001.

Debrabant, A., Bastien, P. and Dwyer, D.M. A unique surface membrane anchored purine-salvage enzyme is conserved among a group of primitive eukaryotic human pathogens. *Mol. Cell. Biochem.* 220: 109-116, 2001.

Robert W. Gwadz, Ph.D.

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Dr. Gwadz received his Ph.D. from the University of Notre Dame in 1970 for studies on the reproductive physiology of mosquitoes. He was a postdoctoral fellow in tropical public health at the Harvard University School of Public Health before joining NIH in 1972. He served as Head of the Medical Entomology Program in LPD until 1995. Dr. Gwadz has worked on the genetic basis of mosquito vector competence and transmission blocking immunity in malaria. He is responsible for administrative management of LPD and for the development and operation of the Malaria Research and Training Center in Bamako, Mali. In recognition of his work in establishing the program on cooperative research on vector-borne diseases in the Middle East, Dr. Gwadz was named an Honorary Fellow of the Hebrew University of Jerusalem, an Honorary Member of the Board of the Ain Shams University (Cairo) Center for Study of Tropical Diseases, and an Honorary Fellow of the Egyptian Society of Parasitologists.

Description of Research Program

Since 1989, LPD has been working with scientists and physicians at the National School of Medicine of Mali in Bamako to develop the Malaria Research and Training Center (MRTC). The Center is now a reality and is a well-equipped, highly productive program where the research is planned, directed, and executed by Malian staff. Programs at the Center are funded by a number of international and U.S. agencies including the World Health Organization (WHO); the International Atomic Energy Agency; the World Bank; U.S. Agency for International Development; the Rockefeller Foundation; the John D. and Catherine T. MacArthur Foundation and the National Institutes of Health. Several American and European universities and research organizations have active research collaborations with MRTC. Dr. Richard Sakai has served as the NIH Resident Scientist at MRTC in Mali since 1990.

LPD activities in Mali include a program for the clinical and field testing of candidate malaria vaccines, studies of the role of hemoglobin C in malaria pathogenesis, and investigations of the molecular basis of malaria parasite resistance to antimalarial drugs. Studies on mosquito vector genetics, cytogenetics, and population biology are a major priority of the Center.

In collaboration with the NIH Office of Minority Health, the Fogarty International Center, and the University of Maryland School of Medicine, the LPD International Research Unit developed a training program for young scientists, medical students, and physicians that permits them to gain experience in an African laboratory or medical school. The program's primary goal is the attraction of underrepresented segments of the U.S. population to careers in tropical medicine, but the program is open to applicants from all backgrounds.

Major Areas of Research

- Anti-malarial drugs
- Malaria vaccines
- Training opportunities in Africa

Selected Recent Publications

Romans, P., Black, W.C. 4th, Sakai, R.K., Gwadz, R.W. Linkage of a gene causing malaria refractoriness to diphenol oxidase-A2 on chromosome 3 of *Anopheles gambiae. Am. J. Trop. Med. Hyg.* 60:22-9, 1999.

Agarwal, A., Guindo, A., Cissoko, Y., Taylor, J.G., Coulibaly, D., Kone, A., Kayentao, K., Djimde, A., Plowe, C.V., Doumbo, O., Wellems, T.E., Diallo, D. Hemoglobin C associated with protection from severe malaria in the Dogon of Mali, a West African population with a low prevalence of hemoglobin S. *Blood* 96:2358-63, 2000.

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Description of Research Program

Plasmodium rRNA genes are unique with regard to copy number, genomic arrangement, and developmental regulation of transcription. One unique feature of *Plasmodium* rRNA genes is the differential expression of different rRNAs at discrete stages of the life cycle. Investigation of this unique system has numerous ramifications for the understanding of this parasitic protozoa. Scientists have used species-specific regions of rRNA to study population dynamics of the parasite in the mosquitoes of an endemic area. Phylogenetic analysis of the rRNA sequences has led to a new and different understanding of the taxonomy of the *Plasmodium* genus and, in particular, to the conclusion that the human pathogen *P. falciparum* is closely related to avian parasites and has not coevolved with its host. Secondary structural analysis of the RNAs has indicated functional differences between developmentally specific ribosomes that involve an alteration of the GTPÕase site. Drug studies indicate that the different ribosomes can be selectively targeted with different antibiotics. This system presents a unique opportunity to investigate translational control in a eukaryotic organism.

The other central project has involved the development of auxotrophic lines of malaria parasites that require supplemental nutrients to grow either in culture or in their natural hosts. The development of auxotrophic lines of bacteria, each with varying requirements as supplements for growth, was an essential tool in defining biochemical pathways in bacterial systems. Along with a general understanding of biochemistry, they have contributed to the definition of new drug targets. More recently the use of nonreverting auxotrophic mutants of *Salmonella* has led to a new generation of live vaccines, some of which have been approved for use. The generation of auxotrophic mutants of malaria parasites potentially holds some of the same promise for parasitology that it held for bacteriology.

Major Areas of Research

- *Plasmodium* rRNA genes
- Development of auxotrophic lines of malaria parasites

Selected Recent Publications

Rathore, D., Wahl, A.M., Sullivan, M., McCutchan, T.F. A phylogenetic comparison of gene trees constructed from plastid, mitochondrial and genomic DNA of *Plasmodium* species. *Mol. Biochem. Parasitol.* 114:89-94, 2001.

Nomura, T., Carlton, J.M., Baird, J.K., del Portillo, H.A., Fryauff, D.J., Rathore, D., Fidock, D.A., Su, X., Collins, W.E., McCutchan, T.F., Wootton, J.C., Wellems, T.E. Evidence for different mechanisms of chloroquine resistance in two *Plasmodium* species that cause human malaria. *J. Infect. Dis.* 183:1653-61, 2001.

Li, J., Collins, W.E., Wirtz, R.A., Rathore, D., Lal, A., McCutchan, T.F. Geographic subdivision of the range of the malaria parasite *Plasmodium vivax*. *Emerg. Infect. Dis.* 7:35-42, 2001.

Rathore, D., McCutchan, T.F. Role of cysteines in *Plasmodium falciparum* circumsporozoite protein: interactions with heparin can rejuvenate inactive protein mutants. *Proc. Natl. Acad. Sci. USA.* 97:8530-5, 2000.

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Description of Research Program

The Section investigates the molecular basis for pathogenesis in malaria with a focus on the events surrounding merozoite invasion of red cells and red cell adhesion to endothelium and placenta. The studies on the molecular basis of *Plasmodium* merozoite invasion of red cells began in my laboratory in 1972 and showed specificity of interaction between merozoites and the red cell. The evidence that Duffy blood group-negative red cells are refractory to invasion by *Plasmodium vivax* opened the way for studies leading to the discovery of parasite and host molecules involved in invasion. The laboratory continues to explore this family of receptors and other molecules involved in the early events of the interaction between merozoites and red cells. We also continue to study human polymorphisms in red cell receptors from malaria-endemic regions that were presumably selected for resistance to disease.

In the early 1980s, my laboratory developed the first assays for evaluating attachment of infected red cells to endothelium and melanoma cell lines—assays that were important for defining the molecular basis of binding. Antibodies to parasite molecules on the infected red cells blocked this binding. As specific molecules were identified by other laboratories (CD36 on macrophages at NYU, ICAM1 on endothelial cells at Oxford, and thrombospondin at the NIH), these were incorporated into CHO cell lines for more specific assays. In 1994, Dror Baruch in Russell Howard's laboratory, the Wellems laboratory, and the Miller laboratory independently identified the molecule, PfEMP1, that was the basis for the two major mechanisms of pathogenesis: antigenic variation and infected red cell adhesion to endothelium and placenta. This family of variant antigens is one focus of research in my laboratory.

Major Areas of Research

- To identify parasite receptors for red cell invasion through bioinformatic analysis of the *Plasmodium* genomic database
- To study these parasite molecules for location in the merozoite and for their role in red cell binding
- To identify extracellular molecules for signaling within the parasite after the merozoite hits a red cell
- Is PfEMP1 involved in binding to CSA in pregnant women?
- Is PfEMP1 involved in binding normal red cells (rosetting)?
- What are the domains of PfEMP1 that bind to four important receptors: CD36, ICAM1, CR1 and CSA? It is necessary to validate each of the binding specificities to determine whether these are indeed the binding domains in the context of the entire PfEMP1 in the red cell membrane?
- Is it possible to produce vaccines against these polymorphic receptor domains on the *var* genes?

Selected Recent Publications

Smith, J.D., Craig, A.G., Kriek, N., Hudson-Taylor, D., Kyes, S., Fagen, T., Pinches, R., Baruch, D.I., Newbold, C.I., and Miller, L.H. Identification of a *Plasmodium falciparum* intercellular adhesion molecule-1 binding domain: a parasite adhesion trait implicated in cerebral malaria. *Proc. Natl. Acad. Sci. USA* 97: 1766-71, 2000.

Buffet, P.A., Gamain, B., Scheidig, C., Baruch, D., Smith, J.D., Hernandez-Rivas, R., Pouvelle, B., Oishi, S., Fujii, N., Fusai, T., Parzy, D., Miller, L.H., Gysin, J., and Scherf, A. *Plasmodium falciparum* domain mediating adhesion to chondroitin sulfate A: a receptor for human placental infection. *Proc. Natl. Acad. Sci. USA* 96: 12743-8, 1999.

Theodore E. Nash, M.D.

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Dr. Nash received his M.D. degree from the University of Miami in 1968 and completed his internship and residency at Duke University. In 1970, he was appointed a Fellow in the Laboratory of Clinical Investigation at NIAID and in 1973 a Staff Fellow in the LPD. After an infectious disease fellowship at the Beth Israel-Children's Hospital in Boston and a fellowship in biological chemistry at Harvard University, he returned to the LPD as a Senior Scientist in 1976. He currently heads the Gastrointestinal Parasites Section.

Description of Research Program

The laboratory is primarily interested in host-parasite interactions of intestinal parasites. A majority of the studies are directed to understanding the cellular and molecular biology and host-parasite interactions of *Giardia lamblia*, a common intestinal parasite that is a cause of diarrhea and other gastrointestinal symptoms all over the world. It is also of interest because it is one of the most primitive eukaryotes. *Giardia* is one of an increasing number of organisms known to undergo surface antigenic variation, a focus of the laboratory since we described the phenomenon. We are trying to determine: the biological role of antigenic variation; whether there is biological selection in addition to immune selection; the molecular mechanisms involved in antigenic variation; how the variant proteins are made and transported to the surface and how are they released from the surface. The variant proteins are very unusual proteins so we are interested in understanding the purpose of certain protein motifs. For instance, these proteins contain a Zn finger motif that is the only recognized Zn finger motif on the surface of any cell or organism. Many studies utilize a mice model of *Giardia* infections.

Microsporidia were originally recognized as causing disease in AIDS, but are now known to cause infections associated with diarrhea in non-immunodeficient persons. The laboratory has defined the proteins that make up the spore wall and determined how the spore wall is made. These proteins are secreted and we are working to devise a diagnostic test using these proteins.

Cysticercosis, an infection with the larval form of the tapeworm, *T.solium*, is a major cause of neurological disease and seizures in the developing world and immigrant populations. We described a newly recognized syndrome characterized by the presence of perilesional edema around dead calcified cysts and usually associated with seizures or other symptoms. Studies in Peru are underway to determine the epidemiology and pathogenesis of this syndrome.

Major Areas of Research

- Cellular and molecular biology of *Giardia* and host-parasite interaction
- Biological purpose of antigenic variation and the role of biological selection vs. immune selection
- Structure-function relationship of the variant surface proteins
- Host-parasite interaction of *Microsporidia*

Selected Recent Publications

Singer, S.M., Elmendorf, H.G., Conrad, J.T., Nash, T.E. Biological selection of variant-specific surface proteins in *Giardia lamblia*. *J. Infect. Dis.* 183: 119-24, 2001.

Nash, T.E., Ohl, C.A., Thomas, E., Subramanian, G., Keiser, P., Moore, T.A. Treatment of patients with refractory giardiasis. *Clin. Infect. Dis.* 33:22-8, 2001.

Yee, J., Mowatt, M.R., Dennis, P.P., Nash, T.E. Transcriptional analysis of the glutamate dehydrogenase gene in the primitive eukaryote, *Giardia lamblia*. Identification of a primordial gene promoter. *J. Biol. Chem.* 275: 11432-9, 2000.

Franklin A. Neva, M.D.

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After medical school at the University of Minnesota, Dr. Neva did his clinical training at the Harvard Medical Service of the Boston City Hospital. He spent 2 years in military service in Cairo, Egypt, and 3 years in research fellowships at Harvard, after which he was on the faculty of the Harvard School of Public Health until 1969. He then came to NIH where he was Chief of the Laboratory of Parasitic Diseases until 1995 at which time he stepped down as Laboratory Chief to devote full time to clinical studies and research.

Description of Research Program

The Opportunistic Parasitic Diseases Section focuses on the immunology and clinical aspects of strongyloidiasis and leishmaniasis, the molecular characterization and identification of major antigens of the parasites which cause these diseases, and the investigation of the circumstances under which these parasites become opportunistic infections. The Section collaborates with international partners in Brazil.

Major Areas of Research

- Immunologic and clinical aspects of strongyloidiasis and leishmaniasis
- Tropical diseases
- Opportunistic parasites

Selected Recent Publications

Carvalho, E.M., Bacellar, O., Porto, A.F., Braga, S., Galvao-Castro, B., Neva, F. Cytokine profile and immunomodulation in asymptomatic human T-lymphotropic virus type 1-infected blood donors. *J. Acquir. Immune Defic. Syndr.* 27:1-6, 2001.

Neva, F.A., Gam, A.A., Maxwell, C., Pelletier, L. Skin test antigens for immediate hypersensitivity prepared from infective larvae of *Strongyloides stercoralis. Am. J. Trop. Med. Hyg.* 65: 567-72, 2001.

Karp, C.L., Neva, F.A. Tropical infectious diseases in human immunodeficiency virus-infected patients. *Clin. Infect. Dis.* 28:947-63, 1999.

Neva, F.A., Filho, J.O., Gam, A.A., Thompson, R., Freitas, V., Melo, A., Carvalho, E.M. Interferon-gamma and interleukin-4 responses in relation to serum IgE levels in persons infected with human T-lymphotropic virus type 1 and *Strongyloides stercoralis. J. Infect. Dis.* 178:1856-9, 1998.

Thomas B. Nutman, M.D.

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Description of Research Program

The Helminth Immunology Section investigates the human host response to helminth parasites—filarial parasites in particular—with the objectives of: 1) developing strategies and methods for intervention in helminth infection; 2) providing insights into the host defense against and the pathogenesis underlying helminth infection; 3) examining the clinical expression of disease and the response to treatment; and 4) understanding the regulation of immune responses associated with immediate hypersensitivity.

The Helminth Immunology Section studies not only the major human filarial diseases—lymphatic filariasis, onchocerciasis, and loiasis—but also is involved in studies of intestinal helminths as well. Because many of these infections occur coincidentally with nonparasitic infections or allergic diatheses, the influence of these infections on mycobacterial diseases, HIV-1 infection, atopy, and responses to orally- or parenterally-delivered vaccines has been a major focus of our research. Further, as none of these helminth infections have good/useful animal models, our work has required significant patient contact, field-based studies (India, West Africa, Ecuador), and the development of *in vitro* methods to study the human response to these important pathogens.

Major Areas of Research

- Regulation of the host immune responses to parasitic helminth infection (primarily filariasis, loiasis, and onchocerciasis)
- Mechanisms of eosinophil activation and eosinophilia
- Control of immediate hypersensitivity reactions
- Influence of helminth infection on expression on nonparasitic infections, atopy and asthma

Selected Recent Publications

Steel, C., and Nutman, T.B. Helminth antigens selectively differentiate unsensitized CD45RA+CD4+ human T cells *in vitro. J. Immunol.* 160:351-360, 1998.

Cooper, P.J., Espinel, I., Paredes, W., Guderian, R.H., and Nutman, T.B. Impaired tetanus specific cellular and humoral responses following tetanus vaccination in human onchocerciasis: a role for IL-10. *J. Infect. Dis.* 178: 1133-1138, 1998.

Marovich, M.A., McDowell, M.A., Thomas, E.K., and Nutman, T.B. Infectious-stage *Leishmania major* induction of IL-12p70 in human dendritic cells is CD40L dependent. *J. Immunol.* 164: 5858-5865, 2000.

Gopinath, R., Ostrowski, M., Justement, S.J., Fauci, A.S., and Nutman, T.B. Filarial infections increase susceptibility to HIV infection in PBMC *in vitro. J. Infect. Dis.* 182: 1804-1808, 2000.

Cooper, P.J., Chico, M., Sandoval, C., Espinel, I., Guevara, A., Kennedy, M.W., Urban, J.F. Jr, Griffin, G.E., and Nutman, T.B. Human infection with *Ascaris lumbricoides* is associated with a polarized cytokine profile. *J. Infect. Dis.* 182: 1207-1213, 2000.

Cooper, P.J., Mancero, T., Sandoval, C., Lovato, R., Guderian, R.H., and Nutman, T.B. Early human infection with *Onchocerca volvulus* is associated with an enhanced parasite-specific cellular immune response. *J. Infect. Dis.* 183: 1662-1668, 2001.

Cooper, P.J., Chico, M., Sandoval, C., Espinel, I., Guevara, A., Levine, M.M., Griffin, G.E., and Nutman, T.B. Human infection with *Ascaris lumbricoides* is associated with suppression of the IL-2 response to recombinant cholera toxin B subunit following vaccination with the live oral cholera vaccine CVD 103-HgR. *Infect. Immun.* 69: 1574-1580, 2001.

José M. C. Ribeiro, Ph.D.

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Dr. Ribeiro obtained his Ph.D. in 1981 from the Biophysics Institute at Federal University of Rio de Janeiro, Brazil, for his study of the role of saliva in feeding in the hematophagous vector of Chagas' disease, *Rhodnius prolixus*. Following postdoctoral studies at Tufts University in Boston, he joined the Department of Tropical Public Health of the Harvard School of Public Health as a Visiting Professor in 1983. In 1985, he was promoted to Assistant Professor and then in 1989 to Associate Professor. In 1990, Dr. Ribeiro joined the Department of Entomology of the University of Arizona as a Professor. In 1996, he joined the LPD as Head of the Medical Entomology Section. His research focuses on the discovery of anticlotting, antiplatelet, vasodilatory, and immunomodulatory substances found in the saliva of bloodsucking insects and ticks. He serves on several editorial boards and is a past Chairman of the Molecular Entomology Steering Committee of the Tropical Diseases Research Program of WHO.

Description of Research Program

The Medical Entomology Section studies the role of vector saliva in blood feeding and is attempting to identify antihemostatic and immunoregulatory substances in the saliva of several arthropods. Ongoing work involves leishmaniasis vectors *Phlebotomus papatasi* and *Lutzomyia longipalpis*, anopheline vectors of malaria, and tick vectors of Lyme disease.

The Section also investigates the role of vector saliva in parasite transmission. In collaboration with the laboratory of Dr. David Sacks, the Section is investigating the role of sand fly saliva in *Leishmania* transmission and in developing novel vaccines targeting vector salivary molecules.

Major Areas of Research

- Medical entomology
- Substances in vector saliva that affect parasite transmission and human immune response

Selected Recent Publications

Ribeiro, J.M., Charlab, R., Valenzuela, J.G. The salivary adenosine deaminase activity of the mosquitoes *Culex quinquefasciatus* and *Aedes aegypti*. *J. Exp. Biol.* 204: 2001-10, 2001.

Valenzuela, J.G., Belkaid, Y., Garfield, M.K., Mendez, S., Kamhawi, S., Rowton, E.D., Sacks, D.L. and Ribeiro, J.M.C. Toward a defined anti-Leishmania vaccine targeting vector antigens. Characterization of a protective salivary protein. *J. Exp. Med.* 194: 331-42, 2001.

Charlab, R., Valenzuela, J.G., Andersen, J., Ribeiro, J.M. The invertebrate growth factor/CECR1 subfamily of adenosine deaminase proteins. *Gene* 267:13-22, 2001.

Belkaid, Y., Valenzuela, J.G., Kamhawi, S., Rowton, E., Sacks, D.L., Ribeiro, J.M. Delayed-type hypersensitivity to *Phlebotomus papatasi* sand fly bite: an adaptive response induced by the fly? *Proc. Natl. Acad. Sci. USA* 97: 6704-9, 2000.

Valenzuela, J.G., Charlab, R., Mather, T.N., Ribeiro, J.M. Purification, cloning, and expression of a novel salivary anticomplement protein from the tick, *Ixodes scapularis*. *J. Biol. Chem.* 275:18717-23, 2000.

Barral, A., Honda, E., Caldas, A., Costa, J., Vinhas, V., Rowton, E.D., Valenzuela, J.G., Charlab, R., Barral-Netto, M., Ribeiro, J.M. Human immune response to sand fly salivary gland antigens: a useful epidemiological marker? *Am. J. Trop. Med. Hyg.* 62:740-5, 2000.

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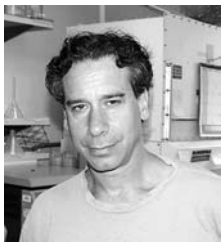

Dr. Sacks obtained his Ph.D. from Harvard University for studies on immune responses to chlamydial infections. Following a postdoctoral fellowship at the National Institute for Medical Research in London (Mill Hill) studying immune suppression in African trypanosomiasis, he joined the Laboratory of Parasitic Diseases in 1980. He became a Senior Investigator in 1986.

Description of Research Program

The Section investigates the cell biology and immunobiology of *Leishmania* parasites and the diseases they produce. The aim of these studies is to develop strategies for treatment and prevention based on new insights regarding the biology of these parasites within their mammalian hosts and sand fly vectors. These insights will in some cases have relevance to diseases caused by other intracellular pathogens, such as tuberculosis, or to other vector-borne diseases, such as malaria.

Understanding the basis of molecular interactions at the sand fly-*Leishmania* interface is fundamental to any study of vector competence and disease transmission. *Leishmania*-sand fly interactions are being studied in the context of the potential barriers to complete development that exist within the midgut of phlebotomine flies, and the parasite-derived molecules that have evolved to overcome these barriers and permit the development of transmissible infections to proceed. The work in the last 4 years has focused on the use of targeted null mutants deficient in expression of cell surface and secreted glycoconjugates to determine their role in parasite survival and development in the fly. Polymorphisms in the phosphoglycan domains of these glycoconjugates have been studied to define their role in controlling species-specific vector interactions. Great emphasis has been placed on developing the first reproducible model of leishmaniasis transmitted by sand fly bite. The primary goal of these studies has been to define the role of vector saliva in modulating the host response to sand fly-transmitted parasites.

Much of the work of the Section involves the establishment and analysis of murine models of the different forms of leishmaniasis seen in humans. Our objective is to develop a natural model of cutaneous leishmaniasis that more closely reproduces the pathology and the innate and adaptive immunity associated with sand fly transmitted infection, and to evaluate protein and DNA-based vaccine candidates in the context of the natural challenge model or sand fly challenge itself. As new vaccines have been developed and their potential demonstrated in the murine models, we have begun to test these vaccines in monkeys, and regard clinical trials as a major program component of the Section in the future.

Major Areas of Research

- Study of molecules from sand fly saliva that modulate the host response to *Leishmania* infection
- Potency and durability of DNA vaccines against leishmaniasis
- Mechanisms of acquired resistance and those controlling persistent infection

Selected Recent Publications

Kamhawi, S., Belkaid, Y., Modi, G., Rowton, E., Sacks, D. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. *Science* 290: 1351-1354, 2000.

Belkaid, Y., Mendez, S., Lira, R., Kadambi, N., Milon, G., Sacks, D. A natural model of Leishmania major infection reveals a prolonged "silent" phase of parasite amplification in the skin before the onset of lesion formation and immunity. *J. Immunol.* 165: 969-977, 2000.

Sacks, D.L., Modi, G., Rowton, E., Spath, G., Epstein, L., Turco, S.J., Beverley, S.M. The role of phosphoglycans in Leishmania-sand fly interactions. *Proc. Natl. Acad. Sci. USA* 97: 406-411, 2000.

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Dr. Xin-zhuan Su obtained his Ph.D. in parasitology from the University of Georgia in 1990. After 2 years of postdoctoral research in the Department of Physiology and Pharmacology, the University of Georgia, he joined the LPD in 1992 as Staff Fellow, Senior Staff Fellow (1994-1997), and Staff Scientist (1997-2000). He was appointed as a tenure-track Investigator in 2000.

Description of Research

The Malaria Genomics Unit uses the malaria parasite genome databases and develops new resources to study the mechanism of drug resistance, gene regulation during parasite sexual development, and parasite population diversity and dynamics. One project in this direction is the genotyping of *Plasmodium falciparum* field isolates with hundreds of microsatellite (MS) markers to identify chromosome loci associated with various drug resistances. This work reveals, in addition to candidate loci for drug resistance, important information on parasite genome structure, diversity, drug selective sweeps, and evolutionary relationships. Another project is the collection of single nucleotide polymorphisms (SNP) from the parasite transporter genes and the association of the SNP to drug responses. Various candidate genes have been identified. We are also developing resources for larger scale studies to include: 1) a genome-wide SNP database with at least one SNP per gene, integrated with MS markers; 2) a worldwide collection of parasite isolates; 3) statistical and computer programs for association analysis specific for the haploid genome of *P. falciparum.* With these resources, we can concentrate on phenotype evaluation and measurement, including parasite responses to various antimalarials and nutrient changes. These tools will also be used to study parasite population genetics and transmission dynamics in endemic areas.

Another research interest is the study of gene function and gene interactions by expressional analysis with microarray and proteomic methods. We are using these techniques to study protein expression in the parasite food vacuole as well as stages of early sexual development (also microarray). Various proteins from the food vacuole and stages of gametocyte development have been identified by 2-D gels and mass spectrometry. Detailed biochemical and genetic characterization will follow after identification of candidate genes.

Major Areas of Research

- *Plasmodium* genetics
- Mechanisms of antimalarial drug resistance
- *Plasmodium* gene regulation and expression

Selected Recent Publications

Su, X., Ferdig, M.T., Huang, Y., Huynh, C.Q., Liu, A., You, J., Wootton, J.C., and Wellems, T.E. A comprehensive genetic map and genome-wide parameters for the human malaria parasite *Plasmodium falciparum. Science* 286, 1351-1353, 1999.

Ferdig, M.T., and Xin-zhuan Su. Microsatellite markers and genetic mapping in *Plasmodium falciparum. Parasitology Today* 16: 307-312, 2000.

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Dr. Wynn obtained his Ph.D. from the University of Wisconsin-Madison Medical School in the Department of Microbiology and Immunology. He is a member of the American Association of Immunologists and the American Society of Tropical Medicine and Hygiene. Dr. Wynn is the recipient of the Oswaldo Cruz Medal and the National Institutes of Health Certificate of Merit.

Description of Research Program

Work in the Schistosomiasis Immunology and Pathology Unit is focused on understanding basic mechanisms of pathogen-induced inflammation as well as vaccine-induced immunity to the helminth parasite, *Schistosoma mansoni.* Transgenic and genetically deficient mice are used as model systems to study host/parasite interactions, with the ultimate goal of designing a highly effective anti-pathology/anti-infection vaccine for this important human disease. Our work in experimental animals is complemented by field-based studies of infected humans conducted primarily in the Northeast of Brazil where schistosomiasis is endemic. Notable accomplishments in recent years have been the development of an experimental vaccine protocol for preventing pathology in schistosomiasis, the identification of IL-13 as the pivotal Th2 cytokine involved in schistosome egg granuloma formation and fibrosis and the discovery that alternatively-activated macrophages play an important immunoregulatory role in the disease. A major emphasis of our current work is on using modern DNA microarray technologies to better understand the major mechanisms/pathways that regulate disease development in schistosomiasis.

Major Areas of Research

- Pathogenesis of schistosomiasis
- Basic mechanisms of inflammation and fibrosis
- Vaccine development

Selected Recent Publications

Chiaramonte, M.G., Schopf, L., Neben, T., Cheever, A.W., Donaldson, D.D., Wynn, T.A. IL-13 is a key regulatory cytokine for T helper 2 cell-mediated pulmonary granuloma formation and IgE responses induced *S. mansoni* eggs. *J. Immunol.* 162: 920-930, 1999.

Hoffmann, K.F., James. S.L., Cheever. A.W., and Wynn, T.A. Studies with double cytokine-deficient mice reveal that highly polarized Th1- and Th2-type cytokine and antibody responses contribute equally to vaccine-induced immunity to *Schistosoma mansoni. J. Immunol.* 163: 927-938, 1999.

Chiaramonte, M.G., Donaldson, D.D., Cheever, A.W., and Wynn, T.A. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper 2-dominated inflammatory response. *J. Clin. Invest.* 104: 777-785, 1999.

Hoffmann, K.F., Cheever, A.W., and Wynn, T.A. IL-10 and the dangers of immune polarization: excessive type-1 and type-2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J. Immunol.* 164: 6406-6416, 2000.

Chiaramonte, M.G., Hesse, M., Cheever, A.W., Wynn, T.A. CpG oligonucleotides can prophylactically immunize against Th2-mediated schistosome egg-induced pathology by an IL-12-independent mechanism. *J. Immunol.* 164: 973-985, 2000.

Hesse, M., Cheever, A.W., Jankovic, D., and Wynn, T.A. NOS-2 mediates the protective anti-inflammatory and anti-fibrotic effects of the Th1-inducing adjuvant, IL-12, in a Th2 model of granulomatous disease. *Amer. J. Pathol.* 157: 945:55, 2000.

Chiaramonte, M.G., Cheever, A.W., Malley, J.D., Donaldson, D.D., Wynn, T.A. Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. *Hepatology* 34: 273-82, 2001.

Laboratory of Persistent Viral Diseases

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Research Activities

The Laboratory of Persistent Viral Diseases (LPVD) of the Rocky Mountain Laboratories is concerned with studies of persistent active or latent virus infections. Attempts are made to manipulate the defense mechanisms of susceptible hosts to increase the frequency or speed of recovery from infection. Investigators place particular emphasis on persistent virus infections of the hemopoietic and lymphoid systems and of the central nervous system. Viral models being examined include HIV and murine retroviruses, rabies virus, and Aleutian disease parvovirus virus of mink. The laboratory is studying the roles of persistent infection in the development of immunosuppression and autoimmune or immune complex disease. Development of DNA and attenuated virus vaccines to prevent infection by retroviruses and rabies virus is also being studied. Transmissible spongiform encephalopathies (TSE diseases or prion diseases) are also under study in LPVD. TSE diseases are being investigated at both the biochemical and biological levels including studies of live animals, infected cell cultures and cell-free generation of the abnormal prion protein. The major research goals of the laboratory are to understand basic pathogenic mechanisms induced by these infections and to study immune or other defense mechanisms used by infected individuals to eliminate such infections.

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Dr. Chesebro received his M.D. degree at Harvard Medical School and did postdoctoral studies in immunology and virology at the Karolinska Institute in Sweden, Stanford University, and the National Institutes of Health. Since 1972 he has worked at the Rocky Mountain Laboratories in Hamilton, Montana, where he is currently Chief of the Laboratory of Persistent Viral Diseases. His research includes studies of retroviral pathogenesis in human and mouse systems and studies of the transmissible spongiform encephalopathies (TSE) or prion diseases.

Description of Research Program

Viral research is aimed at studying pathogenesis and immunity in human and murine retrovirus systems. The immune response involved in protective immunization and spontaneous recovery from Friend murine retrovirus is being analyzed with both conventional mouse genetics and molecular biological approaches to new vaccines. Murine retroviruses and HIV are being studied with a particular focus on pathogenesis of virus-induced brain diseases. The scrapie model of TSE or prion diseases is also being investigated both *in vivo* and *in vitro*, focusing on the multiple roles of prion protein in the disease process.

Major Areas of Research

- Retroviral brain diseases
- Retroviral vaccines and immunity
- Transmissible spongiform encephalopathies or prion diseases

Selected Recent Publications

Chesebro, B. Prion protein and the transmissible spongiform encephalopathy diseases. *Neuron* 24: 503-506, 1999.

Chabry, J., Priola, S.A., Wehrly, K., Nishio, J., Hope, J., Chesebro, B. Species-independent inhibition of abnormal prion protein formation by a peptide containing a conserved PrP sequence. *J. Virol.* 73:6245-6250, 1999.

Peterson, K.E., Iwashiro, M., Hasenkrug, K.J., Chesebro, B. Major histocompatibility complex class I gene controls the generation of gamma interferon-producing CD4+ and CD8+ T cells important for recovery from Friend retrovirus-induced leukemia. *J. Virol.* 74: 5363-7, 2000.

Race, R., Oldstone, M., Chesebro, B. Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. *J. Virol.* 74: 828-33, 2000.

Peterson, K.E., Robertson, S.J., Portis, J.L., and Chesebro, B. Differences in cytokine and chemokine responses during neurological disease induced by polytropic murine retroviruses map to separate regions of the viral envelope gene. *J. Virol.* 75: 2848-2856, 2001.

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Description of Research Program

One project studies Aleutian mink disease parvovirus (ADV) and the pathogenesis of persistent ADV infections. Recent experiments have identified capsid gene residues involved with pathogenicity and *in vivo* replication. Other work has localized regions on the viral capsid that contain neutralizing epitopes. The structure of the ADV capsid has been solved to 22 Angstroms, and this model will facilitate analysis of structure-function interactions at the atomic level.

In addition, we are examining the role of apoptosis in ADV infections. ADV induces caspase-dependent apoptosis, but uniquely, caspase inhibition restricts ADV replication. This effect is probably mediated via caspase cleavage of viral nonstructural genes. A manuscript describing this work is in preparation.

A second project uses adeno-associated virus (AAV)-based gene delivery systems to study transmissible spongiform encephalopathies (TSEs). Prion protein genes and anti-sense reagents in AAV-based vectors will be assessed for their ability to interfere with or modulate TSE infections.

Major Areas of Research

- Aleutian mink disease parvovirus
- Persistent virus infections
- Transmissible spongiform encephalopathies

Selected Recent Publications

Bloom, M.E. Best, S.M., Hayes, S.F., Wells, R.D., Wolfinbarger, J.B., McKenna, R., Agbandje-McKenna, M. Identification of Aleutian mink disease parvovirus (ADV) capsid sequences mediating antibody dependent enhancement of infection, virus neutralization and immune complex formation. *J. Virol.* 75: 11116-27, 2001.

McKenna, R., Olson, N.H., Chipman, P.R., Baker, T.S., Booth, T.F., Christensen, J., Aasted, B., Fox, J.M., Bloom, M.E., Wolfinbarger, J.B., and Agbandje-McKenna, M. Three-dimensional structure of Aleutian mink disease parvovirus: implications for disease pathogenicity. *J. Virol.* 73: 6882-6891, 1999.

Bloom, M.E., Fox, J.M., Berry, B.D., Oie, K.L., and Wolfinbarger, J.B. Construction of pathogenic molecular clones of Aleutian mink disease parvovirus that replicate both *in vivo* and in *vitro*. *Virology* 251: 288-296, 1998.

Oleksiewicz, M.B., Costello, F., Huhtanen, M., Wolfinbarger, J.B., Alexandersen, S., and Bloom, M.E. Subcellular localization of Aleutian mink disease parvovirus proteins and DNA during permissive infection of Crandell feline kidney cells. *J. Virol.* 70: 3242-3247, 1996.

Byron Caughey, Ph.D.

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Dr. Caughey received his Ph.D. degree in biochemistry in 1985 at the University of Wisconsin-Madison for conformational analyses of carboxylic ionophores. After postdoctoral studies at Duke University Medical Center, he moved to Rocky Mountain Laboratories in 1986 to begin work on the transmissible spongiform encephalopathies (prion diseases). He became a tenured Senior Investigator in 1994.

Description of Research Program

Transmissible spongiform encephalopathies (TSEs or prion diseases) such as scrapie, BSE (mad cow disease), Creutzfeldt-Jakob disease, and chronic wasting disease are being studied as models of neurodegenerative protein folding diseases. Emphasis is given to biochemical, conformational, and cell biological studies of the conversion of normal prion protein to the pathogenic, amyloidogenic form. We have developed an *in vitro* conversion reaction that allows us to investigate the pathogenic prion protein transformation under biochemically defined conditions. These studies have provided the first direct evidence that the abnormal, disease-associated prion protein has self-propagating activity, which is a basis prediction of the protein-only hypothesis for the infectious agents of the TSEs. In addition, we have gained insight into the molecular basis for the existence of multiple TSE agent strains and the factors that control whether TSE strains are readily transmitted between different host species. Our primary goals are to determine the mechanism of the prion protein conversion and how it relates to replication of the novel TSE infectious agent. We have also identified new classes of inhibitors of the conversion that serve as therapeutic agents in animals. Our studies suggest strategies for the treatment and diagnosis of TSE diseases in humans and animals.

Major Area of Research

- Transmissible spongiform encephalopathies (prion diseases)
- Protein folding diseases

Selected Recent Publications

Raymond, G.J., Hope, J., Kocisko, D.A., Priola, S.A., Raymond, L.D., Bossers, A., Ironside, J., Will, R.G., Chen, S.G., Petersen, R.B., Gambetti, P., Rubenstein, R., Smits, M.A., Lansbury, P.T. Jr., and Caughey, B. Molecular assessment of the transmissibilities of BSE and scrapie to humans. *Nature* 388: 285-288, 1997.

Caughey, W.S., Raymond, L.D., Horiuchi, M., and Caughey, B. Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. *Proc. Natl. Acad. Sci. USA* 95: 12117-12122, 1998.

Horiuchi, M., Priola, S.A., Chabry, J., and Caughey, B. Interactions between heterologous forms of prion protein: binding, inhibition of conversion, and species barriers. *Proc. Natl. Acad. Sci. USA* 97: 5836-5841, 2000.

Raymond, G.J., Bossers, A., Raymond, L.D., O'Rourke, K.I., McHolland, L.E., Bryant, P.K. III, Miller, M.W., Williams, E.S., Smits, M., and Caughey, B. Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease. *EMBO J.* 19: 4425-4430, 2000.

Wong, C., Xiong, L.-W., Horiuchi, M., Raymond L.D., Wehrly, K., Chesebro, B., and Caughey, B. Sulfated glycans and elevated temperature stimulate PrP^{Sc} dependent cell-free formation of protease-resistant prion protein. *EMBO J.* 20: 377-386, 2001.

Horiuchi, M., Baron, G.S., Xiong, L.W., and Caughey, B. Inhibition of interactions and interconversions of prion protein isoforms by peptide fragments from the C-terminal folded domain. *J. Biol. Chem.* 276: 15489-15497, 2001.

John E. Coe, M.D.

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Description of Research Program

Dr. Coe's previous research involved the characterization and selective regulation of rodent immunoglobulins. His current work is focused on pentraxin proteins (definition, sex hormone regulation) in various hamster species and estrogen induction of hepatocellular carcinoma in the Armenian hamster.

The laboratory is especially interested in a serum protein found in hamsters called female protein (FP). FP is a pentraxin, an ancient and ubiquitous family of proteins that has no known function. FP is a unique pentraxin because hepatic synthesis of FP is regulated by sex hormones so that serum levels in female Syrian hamsters are 200- to 300-fold greater than levels in males. The high serum levels of FP are associated with a shortened longevity of the female hamster due to premature deposits of amyloid. On the other hand, an estrogen-initiated downregulation of FP synthesis in Armenian hamsters is associated with an unusual estrogen-induced hepatotoxicity and neoplastic change in the hamster's liver.

Major Areas of Research

- Pentraxin proteins in various hamster species
- Function of female protein in hamsters

Selected Recent Publications

Melby, P.C., Chandrasekar, B., Zhao, W., Coe, J.E. The hamster as a model of human visceral leishmaniasis: progressive disease and impaired generation of nitric oxide in the face of a prominent Th1-like cytokine response. *J. Immunol.* 166:1912-20, 2001.

Coe, J.E., Race, R.E., Ross, M. J. Serological evidence for an inflammatory response in murine scrapie. *J. Infect. Dis.* 183:185-191, 2001.

Coe, J.E., Vomachka, A.J., Ross, M. J. Effect of hamster pregnancy on female protein, a homolog of serum amyloid P component. *Proc. Soc. Exp. Biol. Med.* 221:369-75, 1999.

Satoh, M.I., Hayes, S.F., Coe, J.E. Estrogen induces cytokeratin aggregation in primary cultures of Armenian hamster hepatocytes. *Cell. Motil. Cytoskeleton* 43:35-42, 1999.

Coe, J.E., Ishak, K.G., Ross, M.J. Estrogen-induced hepatic toxicity and hepatic cancer: differences between two closely related hamster species. *Liver* 18:343-51, 1998.

Leonard H. Evans, Ph.D.

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Dr. Evans received his Ph.D. degree from the Oregon Health Sciences Center in Portland, Oregon, for the study of posttranslational modifications of murine retroviral proteins. Following his graduate work, he did postdoctoral studies at the University of California at Berkeley where he studied the genetic structures of replication-defective transforming retroviruses. He joined the Laboratory of Persistent Viral Diseases in 1980 where he has focused on genetic alterations of retroviruses and their role in disease. The emphasis of his recent work concerns the effect of mixed retrovirus infections on pathology as well as the development of targeted retroviral vectors for application in gene therapy.

Description of Research Program

The major emphasis of the laboratory is the genetic structure of murine leukemia viruses. The current focus is the occurrence and mechanism of genetic alteration in retroviruses and the consequences of such alterations. Ongoing studies include determination of point mutation rates of retroviruses and factors that may influence the mutation rate, the generation of host-range variants of murine leukemia viruses by *in vivo* recombination with host genome, the mechanism of recombination, and the role of such recombinants in disease.

Major Areas of Research

- Genetic alterations of retroviruses and their role in disease
- Retroviral vectors for gene delivery

Selected Recent Publications

Katen, L.J., Januszeski, M.M., Anderson, W.F., Hasenkrug, K.J., Evans, L.H. Infectious entry by amphotropic as well as ecotropic murine leukemia viruses occurs through an endocytic pathway. *J. Virol.* 75: 5018-26, 2001.

Lavignon, M., Richardson, J., Evans, L.H. A small region of the ecotropic murine leukemia virus (MuLV) gag gene profoundly influences the types of polytropic MuLVs generated in mice. *J. Virol.* 71: 8923-7, 1997.

Lavignon, M., and Evans, L.H. A multistep process of leukemogenesis in Moloney murine leukemia virus-infected mice that is modulated by retroviral pseudotyping and interference. *J. Virol.* 70: 3852-3862, 1996.

Kim J. Hasenkrug, Ph.D.

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Dr. Hasenkrug received his Ph.D. from the Albert Einstein College of Medicine and conducted postdoctoral research in the laboratory of Dr. Bruce Chesebro at Rocky Mountain Laboratories. In 1998 he established an independent laboratory to study mechanisms of genetic and vaccine-induced resistance to retroviral disease in a mouse model. A special focus of his research is on the immunological prevention and control of persistent retroviral infections.

Description of Research Program

Scientists in this laboratory use mice infected with a murine leukemia virus called Friend virus to investigate *in vivo* mechanisms of protection from retroviral disease. Such protection can be due either to innate genetic resistance or can be induced by vaccination. Using adoptive transfer experiments in wild type and genetically altered strains of mice, the scientists have identified the specific lymphocyte subsets that are critical for vaccine protection. Current work focuses on determining the specific functions of each critical subset in order to design vaccines best able to elicit protective responses. Of special interest is protection against the establishment of persistent infections because long-term infections with retroviruses can result in serious consequences such as AIDS. Future studies will include following up their recent discovery that persistent Friend virus infections can induce CD4+ regulatory T cells causing suppression of normal immune responses.

Major Areas of Research

- Mechanisms of vaccine protection against retroviral infection
- Persistent retroviral infections: immunology control and immunopathology
- Genetic resistance to retroviral disease

Selected Recent Publications

Iwashiro, M., Messer, R., Peterson, K.E., Stromnes, I.M., Sugie, T., and Hasenkrug, K.J. Immunosuppression by $CD4^+$ regulatory T cells induced by chronic retroviral infection. *Proc. Natl. Acad. Sci. USA* 98: 9226-30, 2001.

Dittmer, U., Peterson, K., Messer, R., Stromnes, I. M., and Hasenkrug, K. J. The role of IL-4, IL-12 and IFNg in primary and vaccine-primed immune responses to Friend retrovirus infection. *J. Virol.* 75: 654-660, 2001.

Iwashiro, M., Peterson, K., Messer, R., Stromnes, I. M., and Hasenkrug, K. J. CD4+ T cells and IFNg in the long-term control of persistent Friend retrovirus infection. *J. Virol.* 75: 52-60, 2001.

Dittmer, U., and Hasenkrug, K. J. Different immunological requirements for protection against acute versus persistent Friend retrovirus infections. *Virology* 272: 177-182, 2000.

Dittmer, U., Brooks, D.M., and Hasenkrug, K.J. Requirement for multiple lymphocyte subsets in protection by a live attenuated vaccine against retroviral infection. *Nature Med.* 5: 189-193, 1999.

Donald L. Lodmell, Ph.D.

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Education: 1961, B.A. Northwestern University; 1963, M.S. University of Montana; 1967, Ph.D. University of Montana.

Military Service: USPHS Commissioned Corps, 1967-1997 (retired).

Honors and Special Scientific Recognition: Phi Sigma; NIH Predoctoral Fellow, 1965-1967; Associate Editor, *Journal of Immunology*, 1979-1987; Faculty Affiliate, University of Montana, 1978-date; USPHS Commendation Medal, 1992; Distinguished Alumnus Award, University of Montana, 1996.

Description of Research Program

Rabies is a successful zoonotic disease persisting over time and achieving worldwide distribution in a variety of warm-blooded species. Major areas of research in our laboratory are to understand the host and viral factors that influence genetically controlled resistance/ susceptibility of inbred strains of mice to rabies virus. We are also asking whether rabies virus persists *in vivo*, and if so, where the virus (genome) is sequestered, and whether it can be activated causing clinical disease. We have become very active in investigating the protective efficacy of recombinant and DNA rabies vaccines. In conjunction with these studies, we are comparing the protective durability (longevity) and efficacy of recombinant and DNA vaccines expressing different or multiple structural proteins of the rabies virus. Additional DNA studies are proceeding with prophylactic DNA vaccination of dogs and cattle, and post-exposure protection of mice and nonhuman primates with DNA vaccines. Most recently we have begun studies involving the transcutaneous, oral and nasal routes of rabies DNA vaccination of mice.

Major Areas of Research

- Rabies viruses
- Rabies DNA vaccines
- Alternative methods/ routes of DNA vaccination

Selected Recent Publications

Lodmell, D.L., Ray, N.B., Parnell, M.J., Ewalt, L.C., Hanlon, C.A., Shaddock, J.H., Sanderlin, D.S., Rupprecht, C.E. DNA immunization protects nonhuman primates against rabies virus. *Nature Med.* 4: 949-952, 1998.

Lodmell, D.L. Rabies DNA vaccines for protection and therapeutic treatment. *Exp. Opin. Invest. Drugs* 8:115-122, 1999.

Lodmell, D.L., Ray, N.B., Ulrich, J.T., Ewalt, L.C. DNA vaccination of mice against rabies virus: effects of the route of vaccination and the adjuvant monophosphoryl lipid A (MPL). *Vaccine* 18: 1059-66, 2000.

Lodmell, D.L. and Ewalt, L.C. Rabies vaccination: comparison of neutralizing antibody responses after priming and boosting with different combinations of DNA, inactivated virus, or recombinant vaccinia virus vaccines. *Vaccine* 18: 2394-2398, 2000.

Lodmell, D.L., Ewalt, L.C. Post-exposure DNA vaccination protects mice against rabies virus. *Vaccine* 19: 2468-2473, 2001.

John L. Portis, M.D.

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Dr. Portis received his M.D. degree in 1971 and completed his internship and residency in pathology at the University of California, Los Angeles. His postdoctoral training in immunology was carried out as a Staff Fellow at the Rocky Mountain Laboratories, and he joined the Laboratory of Persistent Viral Diseases at RML as a Medical Officer when it was formed in 1977. His research focuses on the pathogenesis of neurologic dysfunction caused by infection of the nervous system by murine retroviruses.

Description of Research Program

This laboratory uses molecular biological approaches to study the pathogenesis of noninflammatory spongiform neurodegenerative diseases of mice caused by retroviruses. The goal is to understand, at the molecular level, the mechanisms by which retroviruses cause neuronal injury. Using DNA cloning techniques, researchers are mapping the viral sequences that are determinants of neuroinvasiveness and neurovirulence. Scientists have also identified host factors that influence the neurovirulence of these viruses. Viral nucleic acids and proteins have been localized to specific cell types in the brain; however, neurovirulence is an indirect consequence of virus infection of the brain because the neurons that are damaged are not infected. Recent studies have focused on the characterization of cytokine expression in the brain induced as a consequence of retroviral infection.

Major Areas of Research

- Mechanisms of murine neuronal injury following retroviral infection
- Cytokine expression in brain following murine retroviral infection

Selected Recent Publications

Portis, J.L. Genetic determinants of neurovirulence of murine oncornaviruses. *Adv. Virus Res.* 56:3-38, 2001.

Peterson, K.E., Robertson, S.J., Portis, J.L., Chesebro, B. Differences in cytokine and chemokine responses during neurological disease induced by polytropic murine retroviruses map to separate regions of the viral envelope gene. *J. Virol.* 75: 2848-56, 2001.

Askovic, S., Favara, C., McAtee, F.J., Portis, J.L. Increased expression of MIP-1 alpha and MIP-1 beta mRNAs in the brain correlates spatially and temporally with the spongiform neurodegeneration induced by a murine oncornavirus. *J. Virol.* 75: 2665-74, 2001.

Igietseme, J.U., Portis, J.L., Perry, L.L. Inflammation and clearance of *Chlamydia trachomatis* in enteric and nonenteric mucosae. *Infect. Immun.* 69:1832-40, 2001.

Lynch, W.P., Portis, J.L. Neural stem cells as tools for understanding retroviral neuropathogenesis. *Virology* 271:227-33, 2000.

Askovic, S., McAtee, F.J., Favara, C., Portis, J.L. Brain infection by neuroinvasive but avirulent murine oncornaviruses. *J. Virol.* 74: 465-73, 2000.

Suzette A. Priola, Ph.D.

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Dr. Priola received her Ph.D. degree in microbiology and immunology in 1990 from the University of California, Los Angeles for studies on the molecular mechanisms of herpesvirus latency. She completed her postdoctoral work on the transmissible spongiform encephalopathies with Dr. Bruce Chesebro at the Rocky Mountain Laboratories. Since 1998, she has been a tenure-track Investigator at the Rocky Mountain Laboratories where she continues studying the transmissible spongiform encephalopathies.

Description of Research Program

Our laboratory studies the transmissible spongiform encephalopathies (TSEs or prion diseases). These diseases include Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy (BSE or mad cow disease), and chronic wasting disease in deer and elk. Research in our laboratory focuses on the development of effective anti-TSE therapeutics for these invariably fatal diseases as well as on understanding the molecular basis of TSE disease pathogenesis. Emphasis is placed on pursuing these studies using both *in vitro* and *in vivo* systems. *In vivo*, we have used a transgenic mouse model of TSE disease to identify a potent new class of TSE inhibitors. Our *in vitro* studies are used not only to identify new therapeutic targets and strategies in the development of inhibitors of TSE disease but also to elucidate the basic mechanisms underlying TSE pathogenesis.

We have developed tissue culture based assays to study how the formation of abnormal prion protein (PrP) influences species barriers to TSE infection, TSE strain characteristics, and heritable forms of human TSE disease. Our studies have provided important new insights into the role of the prion protein amino acid sequence and secondary structure in controlling abnormal PrP formation across species barriers and within familial forms of human TSE disease.

Major Areas of Research

- Transmissible spongiform encephalopathies
- Molecular mechanisms of neurodegenerative diseases

Selected Recent Publications

Priola, S.A. and Chesebro, B. Abnormal properties of prion protein with insertional mutations in different cell types. *J. Biol. Chem.* 273: 1180-1185, 1998.

Priola, S., Raines, A., and Caughey, W.S. Porphyrin and phthalocyanine anti-scrapie compounds. Science 287: 1503-1506, 2000.

Priola, S.A. Prion protein diversity and disease in the transmissible spongiform encephalopathies. In: Caughey B, ed. *Advances in Protein Chemistry* 57: 1-27, 2001.

Priola, S.A., Chabry, J., and Chan, K. Efficient conversion of normal prion protein (PrP) by abnormal hamster PrP is determined by homology at amino acid residue 155. *J. Virol.* 75: 4673-4680, 2001.

Vorberg, I., Chan, K., and Priola, S.A. Deletion of β-strand and, α–helix secondary structure in normal prion protein inhibits formation of its protease-resistant isoform. *J. Virol.* 75:10024-32, 2001.

Richard E. Race, D.V.M

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Dr. Race serves as an *ad hoc* reviewer for several scientific journals as well as the FDA Committee on Spongiform Encephalopathies. In addition, he is the Chairman of the Rocky Mountain Laboratories Animal Care and Use Committee. Dr. Race's studies focus on determining factors involved in the interspecies transmission of transmissible spongiform encephalopathies (TSEs) of humans and animals, with emphasis on the influence of prion protein. Pathogenesis of TSEs in their natural hosts is studied extensively.

Description of Research Program

Agents that cause fatal spongiform encephalopathies of animals and humans are unique and have not been well characterized. Scrapie of sheep, bovine spongiform encephalopathy of cattle, and CJD of humans are the best characterized of these TSEs. Experimental evidence suggests that the proteinase K-resistant form of an endogenous protein designated prion protein is important to disease pathogenesis. The laboratory is determining the role of the protein in the pathogenesis. Animal and cell culture systems, including several transgenic mouse models, are being used. Biochemical, molecular, genetic, and biological approaches are all utilized.

Major Areas of Research

- Transmissible spongiform encephalopathies
- Prion protein

Selected Recent Publications

Race, R.E., Priola, S.A., Bessen, R.A., Ernst, D.R., Dockter, J., Rall, G.F., Mucke, L., Chesebro, B., Oldstone, M.B.A. Neuron specific expression of a hamster prion protein minigene in transgenic mice induces susceptibility to hamster scrapie agent. *Neuron* 15: 1183-1191, 1995.

Raeber, A.J., Race, R.E., Brandner, S., Priola, S.A., Sailer, A., Bessen, R.A., Mucke, L., Manson, J., Aguzzi, A., Oldstone, M.B.A., Weissman, C., Chesebro, B. Astrocyte-specific expression of hamster prion protein (PrP) renders PrP knockout mice susceptible to hamster scrapie. *EMBO J.* 16:101-109, 1997.

Race, R., Chesebro, B. Scrapie infectivity found in a resistant species. *Nature* 392: 770, 1998.

Race, R., Jenny, A., Sutton, D. Scrapie infectivity and proteinase K-resistant prion protein in sheep placenta, brain, spleen and lymph node: implications for transmission and antemortem diagnosis. *J. Infect. Dis.* 178: 949-953, 1998.

Race, R., Oldstone, M.B.A., Chesebro, B. Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. *J. Virol.* 74: 828-833, 2000.

Coe, J.E., Race, R.E., Ross, M.J. Serological evidence for an inflammatory response in murine scrapie. *J. Infect. Dis.* 183: 185-191, 2001.

Laboratory of Viral Diseases

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Jonathan W. Yewdell, M.D., Ph.D., Head

DNA Tumor Virus Section
Alison McBride, Ph.D.

Genetic Engineering Section
Bernard Moss, M.D., Ph.D., Head

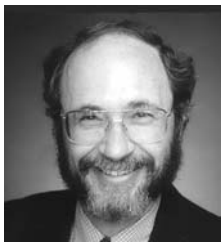
Molecular Genetics Section
Thomas M. Kristie, Ph.D.

Molecular Structure Section
Edward A. Berger, Ph.D., Head

Viral Immunology Section
Jack R. Bennink, Ph.D., Head

Research Activities

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. The studies are designed to increase fundamental knowledge as well as to facilitate the development of new approaches to the prevention and treatment of disease. Current topics of basic research include: viral entry into cells; regulation of gene expression; mechanisms of DNA replication, assembly and transport of viral proteins and particles; actions of viral growth factors and immune defense molecules; determinants of viral virulence; and antigen presentation and viral targets of humoral and cellular immunity. Applied areas of research include development of recombinant expression vectors, candidate vaccines, and antiviral agents. These studies involve a wide range of DNA and RNA viruses including human immunodeficiency virus, poxviruses, herpesviruses, papillomaviruses, and influenza virus. The Laboratory is well equipped with an electron microscope, confocal microscopes, FACS machines, DNA sequencers, PCR machines, a BiaCore apparatus, ultracentrifuges, and other standard items. The members of the Laboratory are interactive and hold weekly seminars in which current research is presented and discussed.



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Dr. Moss received a M.D. from NYU School of Medicine and a Ph.D. in biochemistry from MIT. His research accomplishments include the discovery of the structure and mechanism of synthesis of the capped ends of mRNAs, initial identification of a viral host defense molecule, development of viral expression vectors, and elucidation of many aspects of the replication cycle of poxviruses. Dr. Moss is a member of the National Academy of Sciences, a Fellow of the American Academy of Microbiology, a Fellow of the American Association for the Advancement of Science, and a past president of the American Society for Virology. His awards include the Dickson Prize for Medical Research, the Invitrogen Eukaryotic Expression Award, the ICN Pharmaceuticals International Prize in Virology, the Taylor International Prize in Medicine, and the Bristol-Myers Squibb Award for Distinguished Research in Infectious Disease.

Description of Research Program

Our goals are to determine mechanisms used by viruses to infect cells, express and replicate their genomes, assemble infectious particles, and evade host immune responses. Poxviruses provide excellent model systems that lend themselves to molecular, genetic and microscopic approaches. Basic information obtained is applied to the design of antiviral agents and recombinant vectors for vaccines to prevent HIV and other infections.

Major Areas of Research

- Replication of poxviruses
- Viral immune defense proteins
- Recombinant vaccines

Selected Recent Publications

Senkevich, T.G., White, C.L., Koonin, E.V., and Moss, B. A viral member of the ERV1/ALR protein family participates in a cytoplasmic pathway of disulfide bond formation. *Proc. Natl. Acad. Sci. USA* 97: 12068-12073, 2000.

Ward, B.M., and Moss, B. Visualization of intracellular movement of vaccinia virus virions containing a green fluorescent protein-B5R membrane protein chimera. *J. Virol.* 75: 4802-4813, 2001.

Xiang, Y., and Moss, B. Determination of the functional epitopes of human interleukin-18-binding protein by site-directed mutagenesis. *J. Biol. Chem.* 276: 17380-17386, 2001.

Szajner, P., Weisberg, A.S., Wolffe, E.J., and Moss, B. Vaccinia virus A30L protein is required for association of viral membranes with dense viroplasm to form immature virions. *J. Virol.* 75: 5752-5761, 2001.

Shisler, J.L., and Moss, B. Molluscum contagiosum virus inhibitors of apoptosis: the MC159 v-FLIP protein blocks Fas-induced activation of procaspases and degradation of the related MC160 protein. *Virology* 282: 14-25, 2001.

Calderara, S., Xiang, Y., and Moss, B. Orthopoxvirus IL-18 binding proteins: affinities and antagonist activities. *Virology* 279: 22-26, 2001.

Ishii, K., and Moss, B. Evidence for a role of the vaccinia virus A20R protein in DNA replication determined by clustered charge-to-alanine mutagenesis. *J. Virol.* 75: 1656-1663, 2001.

Earl, PL, Sugiura, W., Montefiori, D.C., Broder, C.C., Lee, S.A., Wild, C., Lifson, J., and Moss, B. Immunogenicity and protective efficacy of oligomeric human immunodeficiency virus type 1 gp140. *J. Virol.* 75: 645-53, 2001.

Amara, R.R., Villinger, F., Altman, J.D. *et al.* Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* 292: 69-74, 2001.

Jack R. Bennink, Ph.D.

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Dr. Bennink obtained his Ph.D. from the University of Pennsylvania for the study of the specificity of virus immune effector T cells. He spent 2 years as a Member of the Basel Institute for Immunology followed by 5 years as Assistant and Associate Professor at the Wistar Institute of Anatomy and Biology before coming to the Laboratory of Viral Diseases in 1987. His research focuses on antigen processing and presentation to class I restricted antiviral T cells.

Description of Research Program

Class I molecules of the major histocompatibility complex (MHC) function to display oligopeptides derived from intracellular proteins to T cells bearing CD8 molecules (T_{CD8+}). T_{CD8+} play a critical role in eradicating intracellular pathogens (particularly viruses) and tumors. On the negative side of the ledger, T_{CD8+} contribute to autoimmunity and rejection of transplanted tissues. Due to the importance of T_{CD8+} in health and disease, considerable effort has gone into delineating the physical nature of the antigen-class I complex and understanding how class I binding peptides are generated and assembled with class I molecules ("antigen processing"). There is now Ångström-level resolution of class I-peptide complexes and a broad outline of the principal class I antigen processing pathways. Many important questions regarding class I antigen processing remain, particularly those concerned with how class I binding peptides are generated. Armed with knowledge regarding antigen processing and presentation gleaned from *in vitro* systems combined with novel reagents that enable detection of specific class I-peptide complexes and T_{CD8+} specific for these complexes, it is now possible to tackle important questions regarding the *in vivo* generation of anti-viral T_{CD8+} responses.

The broad objectives of the Cellular Biology and Viral Immunology Sections are to: 1) use the sensitive tools of cellular immunology to discover and define basic cellular processes; and 2) improve our capacity to control beneficial and deleterious T_{CD8+} responses by gaining a fundamental understanding of the underlying cellular events that lead to presentation of antigens to T_{CD8+}, and the regulation of T_{CD8+} responses following a viral infection.

Major Areas of Research

- Antigen presentation and processing
- MHC class I molecules

Selected Recent Publications

Schubert, U., Anton, L.C., Gibbs, J., Norbury, C.C., Yewdell, J.W., Bennink, J.R. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 404:770-4, 2000.

Chen, W., Norbury, C.C., Cho, Y., Yewdell, J.W., Bennink, J.R. Immunoproteasomes shape immunodominance hierarchies of antiviral CD8 (+) T cells at the levels of T-cell repertoire and presentation of viral antigens. *J. Exp. Med.* 193:1319-26, 2001.

Bennink, J.R. A novel vaccine approach to stimulate class I restricted T cells. *J. Immunother.* 23:611-2, 2001.

Prasad, S.A., Norbury, C.C., Chen, W., Bennink, J.R., Yewdell, J.W. Cutting edge: recombinant adenoviruses induce CD8 T cell responses to an inserted protein whose expression is limited to nonimmune cells. *J. Immunol.* 166:4809-12, 2001.

Norbury, C.C., Princiotta, M.F., Bacik, I., Brutkiewicz, R.R., Wood, P., Elliott, T., Bennink, J.R., Yewdell, J.W. Multiple antigen-specific processing pathways for activating naive CD8+ T cells in vivo. *J. Immunol.* 166:4355-62, 2001.

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Description of Research Program

My laboratory has had a long-standing interest in how enveloped viruses enter target cells, and in using our knowledge to devise novel approaches to treat and prevent viral diseases. Our early studies involved structure/function analysis of the HIV Env-CD4 interaction. Using specialized approaches, we then discovered the first HIV coreceptors, CXCR4 (fusin) and CCR5, as well as several minor coreceptors. We are probing the mechanisms by which Env interaction with CD4 and coreceptor triggers membrane fusion and virus entry. Our studies indicate that Env first binds to CD4, then undergoes a conformational change that exposes conserved determinants involved in binding to coreceptor. These insights enabled us to devise a novel chimeric protein with very potent and broad neutralizing activity against HIV-1. We are exploring the potential use of this agent as an immunotherapeutic to prevent HIV infection during acute exposure (topical microbicide, maternal-newborn transmission, post-exposure prophylaxis). A second applied direction is an extension of our earlier work on hybrid proteins containing CD4 or anti-Env antibodies linked to effector domains of the cytotoxic protein *Pseudomonas* exotoxin A. We are collaborating to assess the potential utility of such agents for eliminating the infected cell reservoirs that persist in HIV-infected persons despite effective antiretroviral therapy. Our research interests have expanded to other enveloped viruses of clinical significance, with the goals of identifying critical cellular receptors, elucidating virus entry mechanisms, and developing novel treatment and prevention strategies. We recently identified CD46 as the cellular receptor for human herpesvirus-6.

Major Areas of Research

- Mechanisms of viral Env glycoprotein-receptor interactions (HIV, herpesviruses, flaviviruses, etc.)
- Novel treatment and prevention strategies based on viral Env glycoprotein/receptor interactions

Selected Recent Publications

Berger, E.A., Murphy, P.M. and Farber, J.M. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Ann. Rev. Immunol.* 17:657-700, 1999.

Santoro, F., Kennedy, P.E., Locatelli, G., Malnati, M.S., Berger, E.A., and Lusso, P. CD46 is a cellular receptor for human herpesvirus 6. *Cell* 99: 817-827, 1999.

Salzwedel, K., Smith, E.D., Dey, B. and Berger, E.A. Sequential CD4/coreceptor interactions in human immunodeficiency virus type 1 Env function: soluble CD4 activates Env for coreceptor-dependent fusion and reveals blocking activities of antibodies against cryptic conserved epitopes on gp120. *J. Virol.* 74:326-333, 2000.

Goldstein, H., Pettoello-Mantovani, M., Bera, T.K., Pastan, I. and Berger, E.A. Chimeric toxins targeted to the HIV-1 envelope glycoprotein augment the in vivo activity of highly active antiretroviral therapy in SCID-hu mice. *J. Infect. Dis.* 181:921-926, 2000.

Salzwedel, K. and Berger, E.A. Cooperative subunit interactions within the oligomeric Env of HIV-1: functional complementation of specific defects in gp120 and gp41. *Proc. Natl. Acad. Sci. USA* 97:12794-12799, 2000.

Thomas M. Kristie, Ph.D.

Senior Investigator, LVD

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B.S. Biology, 1981, Fairfield University; Ph.D., 1986, Committee on Virology, University of Chicago; Postdoctoral Fellow, 1986-7, Committee on Virology, University of Chicago; Postdoctoral Fellow, 1987-93, Center for Cancer Research, Massachusetts Institute of Technology; 1993-present, LVD, NIAID.

Description of Research Program

After a primary infection with herpes simplex virus (HSV), the virus remains latent in the sensory neurons of the individual until complex stimuli such as stress, hormonal alterations, or tissue damage results in reactivation of viral replication. The resulting disease can range from mild recurrent lesions to more significant and life-threatening encephalitis. The laboratory focuses upon the identification of critical components that regulate the first stage of viral gene expression (IE genes) during lytic phase replication as well as signals that result in reactivation from latency.

The lytic replication cycle of HSV has been intensely studied. The genes of HSV are transcribed in an ordered manner beginning with the induction of the immediate early (IE) genes. These genes are controlled by a complex regulatory enhancer domain consisting of elements that respond to both viral and cellular transcription factors and nucleate the assembly of multiprotein complexes. The critical component of the IE enhancer core complex is the cellular C1 factor. This protein is a unique transcription factor produced as a 230 Kd protein and proteolytically processed into a family of amino and carboxyterminal polypeptides. Studies in the laboratory address the role and biochemical mechanisms of the C1 factor and associated proteins in the latency-reactivation process as well as the development of animal model systems for analyzing these proteins *in vivo.*

A second area of study is the analysis of site-specific proteolytic processing. Specific proteolysis plays a significant role in the regulation of many basic biological processes. Proteolysis of the C1 factor results in the generation of a family of related polypeptides. Several models for the biological role of this processing include the regulation of transcriptional activity, the regulation of protein subcellular localization, and the regulation of protein-protein interactions.

Major Areas of Research

- Herpes simplex virus
- Gene expression/transcription
- Site-specific proteolysis

Selected Recent Publications

Kristie, T.M., Vogel, J.L., and Sears, A.E. Nuclear localization of the C1 factor (HCF) in sensory neurons correlates with initiation of reactivation of HSV from latency. *Proc. Natl. Acad. Sci.USA* 96: 1229-1233, 1998.

Vogel, J. and Kristie, T.M. The novel coactivator C1 (HCF) coordinates multiprotein enhancer formation and mediates transcription activation by GABP. *EMBO J* .19: 683-690, 2000.

Vogel, J.L. and Kristie, T.M. Autocatalytic proteolysis of the transcription factor-coactivator C1 (HCF): a potential role for proteolytic regulation of coactivator function. *Proc. Natl. Acad. Sci. USA* 97: 9425-9430, 2000.

Alison McBride, Ph.D.

Senior Investigator, LVD

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Alison McBride received a B.Sc. in molecular biology from the University of Glasgow, Scotland in 1981and a Ph.D. in biochemistry from the Imperial Cancer Research Fund and University of London, England in 1986. Dr. McBride studied as a postdoctoral fellow in the National Cancer Institute and has been an Investigator in the Laboratory of Viral Diseases in NIAID since 1994.

Description of Research Program

The papillomaviruses are small DNA viruses that induce persistent benign epithelial lesions. In some cases these lesions can progress to malignant carcinomas, the most notable of which is cervical cancer. The viral E1 and E2 genes regulate viral transcription and replication, and our objective has been to understand the mechanisms by which they control the viral life cycle. My laboratory studies the ways in which the E2 proteins regulate viral gene expression and how the E1 and E2 proteins act in concert to replicate papillomavirus DNA. These studies have provided detailed information about how the proteins interact with the viral DNA and with each other and how their activities are regulated within infected cells. The E2 transactivator protein is required for viral transcriptional regulation, DNA replication and stable episomal maintenance of viral genomes. We have shown that the E2 proteins maintain and segregate the papillomavirus genomes in persistently infected cells by linking them to mitotic chromosomes.

Papillomaviruses induce proliferative epithelial lesions and can only undergo vegetative replication in terminally differentiated keratinocytes. This has hampered the study of the complete viral life cycle because of the difficulties in generating a differentiated stratified epithelium in tissue culture. Using a system of organotypic raft cultures and xenografts on nude mice, we can generate fully differentiated epithelium in which papillomaviruses can replicate and produce infectious viral particles. This system is being used for analysis of the roles of the E1 and E2 viral gene products in the complete viral life cycle.

In the majority of carcinomas, papillomavirus genomes are integrated into cellular chromosomes such that the E1 and/or E2 genes are disrupted. This finding has led to the hypothesis that disruption of the E1 and E2 regulatory functions is a critical step in progression to a carcinoma. We are studying the role of the E1 and E2 regulatory functions in keratinocyte growth and differentiation. Because the E1 and E2 proteins have both positive and negative effects on the viral life cycle and on malignant progression, a detailed understanding of their regulatory mechanisms is crucial for the design of antiviral drugs and strategies.

Major Areas of Research

- Papillomaviruses
- DNA replication and genome segregation
- Keratinocyte biology

Selected Recent Publications

Penrose, K.J. and McBride, A.A. Proteasome-mediated degradation of the papillomavirus E2-TA protein is regulated by phosphorylation and modulates viral genome copy number. *J. Virol.* 74 :6031-6038, 2000.

McBride, A.A., Dlugosz, A. and Baker, C.C. Production of infectious bpv-1 virions from cloned viral DNA using an organotypic raft /xenograft technique. *Proc. Natl. Acad. Sci. USA* 97: 5534-5539, 2000.

McBride, A.A. and Khleif, S.N. Antiviral strategies for the treatment of cervical cancer. *International Antiviral News* 8: 113-117, 2000.

Jonathan W. Yewdell, M.D., Ph.D.

Head, Cellular Biology Section, LVD

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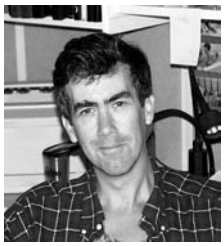

Dr. Yewdell received an A.B. in biochemistry from Princeton University in 1975, writing his undergraduate thesis with Dr. Arnold Levine on immune recognition of virus-transformed cells. He received an M.D. and a Ph.D. in immunology from the University of Pennsylvania in 1981, working with Dr. Walter Gerhard on the generation and characterization of antiviral monoclonal antibodies. As a postdoctoral fellow, he worked with Dr. David Lane at the Imperial College, London, United Kingdom, studying the newly discovered p53 protein. From 1983 to 1987, he was Assistant Professor at the Wistar Institute in Philadelphia. In 1987, Dr. Yewdell joined LVD and in 1993 was asked to lead the Cellular Biology Section. His research focuses on the *in vitro* and *in vivo* generation of antigenic peptides for recognition by CD8+ T cells, with occasional forays into basic cell biology.

Description of Research Program

Class I molecules of the major histocompatibility complex (MHC) function to display oligopeptides derived from intracellular proteins to T cells bearing CD8 molecules (T_{CD8+}). T_{CD8+} play a critical role in eradicating intracellular pathogens (particularly viruses) and tumors. On the negative side of the ledger, T_{CD8+} contribute to autoimmunity and rejection of transplanted tissues. Due to the importance of T_{CD8+} in health and disease, considerable effort has gone into delineating the physical nature of the antigen-class I complex and understanding how class I binding peptides are generated and assembled with class I molecules ("antigen processing"). There is now Ångström-level resolution of class I-peptide complexes and a broad outline of the principal class I antigen processing pathways. Many important questions regarding class I antigen processing remain, particularly those concerned with how class I binding peptides are generated. Armed with knowledge regarding antigen processing and presentation gleaned from *in vitro* systems combined with novel reagents that enable detection of specific class I-peptide complexes and T_{CD8+} specific for these complexes, it is now possible to tackle important questions regarding the *in vivo* generation of anti-viral T_{CD8+} responses.

The broad objectives of Cellular Biology and Viral Immunology Sections are to: 1) use the sensitive tools of cellular immunology to discover and define basic cellular processes; and 2) improve our capacity to control beneficial and deleterious T_{CD8+} responses by gaining a fundamental understanding of the underlying cellular events that lead to presentation of antigens to T_{CD8+}, and the regulation of T_{CD8+} responses following a viral infection.

Major Areas of Research

- Antigen presentation and processing
- MHC class I molecules

Selected Recent Publications

Schubert, U., Ott, D.E., Chertova, E.N., Welker, R., Tessmer, U., Princiotta, M.F., Bennink, J.R., Krausslich, H.G., Yewdell, J.W. Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. *Proc. Natl. Acad. Sci. USA* 97:13057-62, 2000.

Prasad, S.A., Norbury, C.C., Chen, W., Bennink, J.R., Yewdell, J.W. Cutting edge: recombinant adenoviruses induce CD8 T cell responses to an inserted protein whose expression is limited to nonimmune cells. *J. Immunol.* 166:4809-12, 2001.

Norbury, C.C., Princiotta, M.F., Bacik, I., Brutkiewicz, R.R., Wood, P., Elliott, T., Bennink, J.R., Yewdell, J.W. Multiple antigen-specific processing pathways for activating naive CD8+ T cells *in vivo*. *J. Immunol*. 166:4355-62, 2001.

Princiotta, M.F., Schubert, U., Chen, W., Bennink, J.R., Myung, J., Crews, C.M., Yewdell, J.W. Cells adapted to the proteasome inhibitor 4-hydroxy-5-iodo-3-nitrophenylacetyl-Leu-Leu-leucinal-vinyl sulfone require enzymatically active proteasomes for continued survival. *Proc. Natl. Acad. Sci. USA* 98:513-8, 2001.

Malaria Vaccine Development Unit

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Carole Long, Ph.D.

Process Development
Allan Saul, Ph.D.
Anthony Stowers, Ph.D.

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Research Activities

The Malaria Vaccine Development Unit (MVDU) is a new NIAID initiative to respond to the global need for a vaccine against malaria. The MVDU is focusing primarily on recombinant proteins derived from asexual blood stages and sexual stages of malaria parasite development. Immunity to the blood stages would primarily be designed to reduce pathology associated with malaria, while immunity to the sexual stages would be designed to reduce transmission via the mosquito vector. To accomplish these goals, the MVDU has facilities for protein expression in a variety of recombinant systems as well as subsequent purification and analysis. Once produced and purified, blood-stage antigens are tested by immunization and challenge studies in *Aotus* monkeys to identify the most promising candidates. Antigens derived from sexual stages are being evaluated by their capacity to elicit antibodies that inhibit transmission to mosquitoes in membrane feeding experiments. Other aspects of the vaccine development pathway include determination of optimal formulation of antigen and adjuvants in rhesus monkeys, identification of immunologic assays that correlate with protective immunity, exploration of synergistic responses to different parasite antigens, and the execution of human clinical trials.

The most advanced blood-stage vaccine candidate within the MVDU is the merozoite surface protein-1 (MSP-1) of *Plasmodium falciparum*. The MVDU has been evaluating a 42kDa carboxy-terminal portion of this molecule produced in a baculovirus expression system by collaborators at Novavax. Two recent studies have shown that *Aotus nancymai* monkeys can be protected against a *P. falciparum* challenge infection after immunization with this antigen. We anticipate that clinical grade material will be produced for a Phase I clinical trial at the NIH Clinical Center. In addition, other recombinant systems are being compared to obtain this antigen in a correct conformation.

Transmission blocking vaccines have several possible roles as part of a multi-faceted strategy directed to eradication of parasites from a low-transmission area or as a means of protecting a vaccine or drug directed at pre-erythrocytic or erythrocytic stages. It is known that antibodies are the primary mediators of this immunity. In addition, this system has another advantage since an *in vitro* biological assay is available. This involves *in vitro* mixing of sexual stage parasites (gametocytes) with specific antisera and subsequent feeding to anopheline mosquitoes. After a suitable period of development, oocysts in the mosquitoes are enumerated and compared with those mosquitoes receiving control sera. The most advanced transmission blocking vaccine candidate within the MVDU is a sexual stage antigen of *P. falciparum* (Pfs25) and its homologue in *P. vivax* (Pvs25). Identification of a process for expression and purification of both these proteins is nearly completed, and both polypeptides will be produced as clinical grade material in recombinant *Saccharomyces cerevisiae* through a collaborative arrangement with DMID at the Forest Glen facility of Walter Reed.

The clinical trials component anticipates doing many of the Phase 1 clinical studies in the NIH Clinical Center. Assuming antigen formulations are found to be safe and immunogenic, further Phase 1 and Phase 2 testing will be done in Mali or other suitable field sites.

Molecular and Cellular Immunogenetics Section

Thomas J. Kindt, Ph.D., Head
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Dr. Kindt received his Ph.D. in biochemistry from University of Illinois at Urbana. After postdoctoral training at the City of Hope in Duarte, CA he joined The Rockefeller University in New York. In 1977, he was appointed Chief of the Lab of Immunogenetics, NIAID, a position he held until 1998. He is currently Head of the Molecular and Cellular Immunogenetics Section and Director of the Division of Intramural Research, NIAID.

Research Activities

The Molecular and Cellular Immunogenetics Section studies the interactions between retroviral pathogens and the host. Studies at the molecular and cellular level attempt to probe the mechanisms by which infections with human T-cell lymphotropic virus type 1 (HTLV-1) lead to aggressive and fatal leukemia in a fraction of infected individuals but cause asymptomatic infection in most cases. Recent studies use genetically engineered viral mutants to determine effects of virus proteins on cellular functions including signal transduction. *In vivo* studies utilize a rabbit model for T-cell leukemia that closely mimics human disease. Like the human, the rabbit responds variably to HTLV-1 infection and a spectrum of disease conditions has been observed. Comparisons of virus and cells from asymptomatic infected animals with virus and cells from animals with leukemia seek to determine what interplay of viral and host factors gives rise to these diverse outcomes of infection with a single retrovirus. Studies of molecular clones of the virus aim to determine the impact of substitutions in individual viral genes on the disease process. In addition, detailed phenotypic analyses of leukemia cells aim to identify host cell responses to infection that may influence the outcome of the infection process.

Selected Recent Publications

Mahana, W., Zhao, T.M., Teller, R., Robinson, M.A. and Kindt, T.J. Genes in the pX region of human T cell leukemia virus 1 influence vav phosphorylation in T cells. *Proc. Natl. Acad. Sci. USA* 95: 1782-1787, 1998.

Mahana, W., Samaan, A., Zhao, T-M., Kindt, T.J., and Simpson, R.M. Evidence for humoral and cellular reactivity against keratin and thyroglobulin in HTLV-1-infected rabbits. *Autoimmunity* 32: 57-65, 2000.

Kindt, T.J., Said, W. A., Bowers, F. S., Mahana, W., Zhao, T-M., and Simpson, R.M. Passage of human T cell leukemia virus type-1 during progression to cutaneous T-cell lymphoma results in myelopathic disease in an HTLV-1 infection model. *Microbes and Infection* 2: 1139-46, 2000.

Cao, F., Ji, Y., Huang, R., Zhao, T., Kindt, T.J. Nucleotide sequence analyses of partial envgp46 gene of human T-lymphotropic virus type I from inhabitants of Fujian Province in Southeast China. *AIDS Res. Hum. Retroviruses* 16:921-3, 2000.

Fain, M.A., Zhao, T., Kindt, T.J. Improved typing procedure for the polymorphic single-copy RLA-DQA gene of the rabbit reveals a new allele. *Tissue Antigens* 57: 332-8, 2001.

Research Technologies Branch

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Carl Hammer, Ph.D.
Jan Lukszo, Ph.D.
Mark Garfield

Research Technologies Development Section
Mary Ann Robinson, Ph.D.

Transgenic/Knockout Mouse Facility
Judy Hewitt, Ph.D.

Research Support Activities

The Research Technologies Branch (RTB) provides state-of-the-art technologies and applications to the DIR through internal development and external collaborations. The Branch operates core facilities and consults with investigators on specific projects and provides training on the latest instrumentation. The RTB recently added a Microarray Research Facility and a Technology Development Section to complement the four established sections: Biological Imaging Facility, Flow Cytometry Section, Protein Chemistry Section and the Transgenic/Knockout Mouse Facility.

The Biological Imaging Facility provides instrumentation and technical expertise in the fields of confocal, fluorescence, and live-cell video microscopy. The facility also provides assistance on image analysis, 3D reconstruction, quantification, time-lapse animation, and presentation of data. The Flow Cytometry Section provides cytometric sorting and analysis services. Instrumentation includes 3 multi-parameter cell sorters, 2 user-operated cytometers, and a 3-laser bench-top analyzer. The FCS also provides an introductory course in flow cytometry and individualized consultation on specific projects. The Microarray Research Facility is currently producing human, mouse, and TB whole genome arrays and will focus on genome arrays from other infectious disease-causing organisms as the information becomes available. The Protein Chemistry Section provides expertise and services in a wide array of analytical instrumentation, with emphasis on protein sequence and mass spectrometry analytical services and peptide synthesis support with rigid quality control. The Research Technologies Development Section works with the other sections to develop new applications, especially those that span several technology platforms. The Transgenic/Knockout Mouse Facility provides the DIR with a variety of cutting edge technologies to produce, propagate and preserve genetically engineered mice and facilitate research using the mice. Services include transgenic production, ES cell manipulations and knockout production, breeding, embryo and sperm cryopreservation, *in vitro* fertilization, re-derivation, ovary transplantation and speed congenics.

In addition to the six sections, the Branch is establishing a bioinformatics infrastructure to support the genomics and proteomics programs.

Rocky Mountain Microscopy Branch

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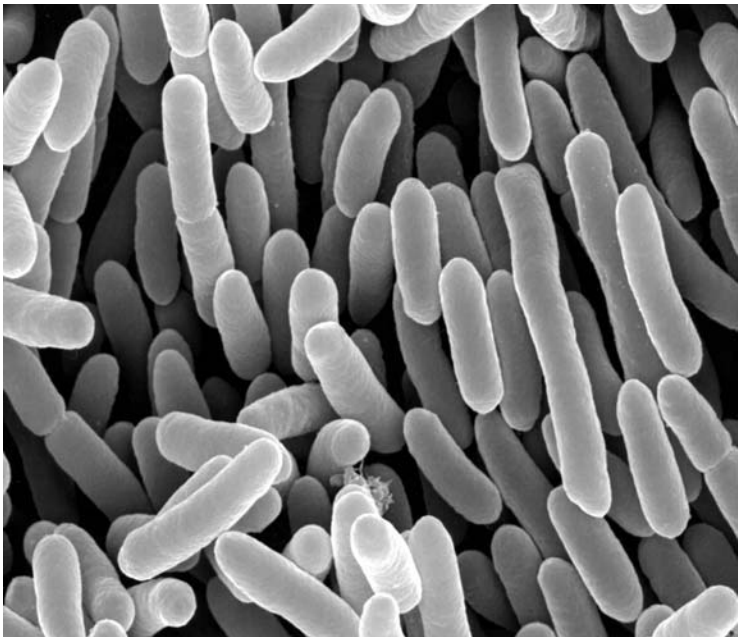

Research Activities

The Rocky Mountain Laboratories Microscopy Branch (RMMB) has both a well-developed research program and an active support facility function. As part of its research function, the Branch investigates the molecular structure and function of genes and gene products that are important in microbial pathogenesis. Although a multidisciplinary approach is used to study various aspects of the disease process, important human pathogens such as those causing Lyme disease are emphasized. The Branch's research laboratories use modern molecular biology techniques as well as microscopy methods to study microbial pathogens and their hosts, attempting to define in molecular terms important features of the host-pathogen relationship. The Branch seeks to apply detailed molecular information about virulence determinants for the development of improved diagnostic techniques, effective vaccines or both. A central and important component of the Rocky Mountain Laboratories Microscopy Branch is the microscopy core facility. As part of its support facility function, RMMB provides NIAID researchers with up-to-date microscopy support including laser scanning confocal microscopy, fluorescence microscopy, laser trapping and microdissection, transmission electron microscopy, field emission scanning electron microscopy and associated ultramicrotomy, cryomicrotomy, plasma etch, immunoelectron microscopy protocols, with image analysis, photographic and digital electronic image archiving and internet image transmission capabilities. The core facility emphasizes the effective interaction of skilled and knowledgeable microscopists with researchers throughout the institute covering a broad range of biomedical research interests. Central to this interaction is not only the latest and most up-to-date techniques and experimental approaches, but the willingness by core facility staff to keep abreast of modern developments and to apply those appropriately to a given research project.

Claude F. Garon, Ph.D.

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Dr. Garon received his Ph.D. in microbiology from Georgetown University. In addition to serving as Branch Chief and Section Head, he is a faculty affiliate in the Division of Biological Sciences and the Department of Pharmaceutical Sciences, The University of Montana, and a member of the Advisory Committee, University of Montana Electron Microscopy Facility. Dr. Garon has received the NIH Award of Merit and the NIH Director's Award, and served as Chairman of the Board of Directors, Center of Excellence in Biotechnology, Montana Science and Technology Alliance. Dr. Garon served as Chairman, Montana Governor's Focus Group on Biotechnology and as a member of the Governor's Task Force for Research and Development and of the Board of Directors of the International Heart Institute of Montana. He has served as a member of the editorial boards of the *Journal of Clinical Microbiology* and the *Journal of Spirochetal and Tick-Borne Diseases.*

Description of Research Program

Since Lyme disease is the most common arthropod-borne infection in the United States, the laboratory has continued its molecular dissection of the Lyme disease spirochete, *Borrelia burgdorferi*, which began with its discovery as causative agent by Dr. Willy Burgdorfer, now an active Scientist Emeritus in our Branch. The disease is a multi-system disorder with dermatologic, neurologic and rheumatologic manifestations. *Borrelia burgdorferi* alternates between the distinct microenvironments of the tick vector and a mammalian host. When the microbe is transmitted from the tick vector to its host, the bacterium experiences significant alterations in both temperature and pH. Previously we documented numerous alterations in the membrane protein profiles when *B. burgdorferi* was grown at pH 7.0 compared to pH 8.0. We have subsequently identified 11 genes localized to linear plasmids that are regulated by the *in vitro* pH. Some genes were indirectly identified by Matrix Assisted Ionization Desorption Time of Flight (MALDI-TOF) analysis of proteins separated by 2D-NEPHGE that were synthesized in greater amounts when spirochetes were grown at pH 7.0 versus pH 8.0. Other genes were identified by screening a *B. burgdorferi* library with cross-absorbed, hyperimmune rabbit serum. All 11 genes were transcriptionally regulated, yet the degree of pH regulation observed in cultures grown at pH 7.0 compared to pH 8.0 varied, where some genes were more tightly regulated than others. The regulation of genes in response to the change in pH may aid *B. burgdorferi* in adapting effectively to either the tick or mammalian environment. Researchers in the Section continue to examine these and other characteristics of the causative agent and its interaction with host cells with the ultimate aim of improving the prevention, treatment, and diagnosis of Lyme disease.

Major Areas of Research

- Microscopy
- Microbial pathogenesis

Selected Recent Publications

Carroll, J.A., Garon, C.F., and Schwan, T.G. Effects of environmental pH on membrane proteins in *Borrelia burgdorferi. Infection and Immunity* 67: 3181-3187, 1999.

Carroll, J.A., Cordova, R.M., and Garon, C.F. Identification of eleven pH-regulated genes in *Borrelia burgdorferi* localizing to linear plasmids. *Infection and Immunity* 68: 6677-6684, 2000.

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH

National Institute of Allergy and Infectious Diseases
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