Neurovirology Research Fellowships

The program director is Dr. Donald Gilden. Dr. Gilden completed his neurology residency at the University of Chicago in 1967 under the direction of two superb clinical neurologists: Drs. Richard Richter (adult neurology) and Douglas Buchanan (child neurology). After residency, Dr. Gilden was a major in the U.S. Army Medical Corps where he served as a staff neurologist at Walter Reed Army Medical Center from 1967-69 during the Vietnam War. From 1969-71, Dr. Gilden was an NIH postdoctoral fellow at Johns Hopkins University under the mentorship of Dr. Neal Nathanson.

Dr. Gilden is board certified in neurology, has been an examiner many times for the American Board of Neurology and Psychiatry, and has been training neurology residents and teaching medical students for more than 35 years.

In 1971, Dr. Gilden joined the faculty of the Department of Neurology at the University of Pennsylvania (Dr. Lewis P. Rowland, Chair) and the Wistar Institute (Dr. Hilary Koprowski, Director). In 1980, Dr. Gilden became Full Professor at the University of Pennsylvania and the Wistar Institute. In 1985, Dr. Gilden left Philadelphia to become Professor and Chair of the Department of Neurology with a joint appointment in the Department of Microbiology at the University of Colorado Denver.

Dr. Gilden's entire scientific career has been devoted to the study of neurotropic virus infection. His scope of research has spanned the immunopathology of arenavirus (both lymphocytic choriomeningitis and Tamiami virus) infection of the CNS, the relationship of parainfluenza virus (both 6/94 and canine distemper virus) persistence in the nervous system to demyelination, the molecular pathogenesis of human herpesvirus (both herpes simplex and varicella zoster virus) latency, and a comprehensive search for virus genetic material in multiple sclerosis brain.

Dr. Gilden has served as deputy director of a neurovirology training grant at the University of Pennsylvania School of Medicine and has been director of this highly successful training grant in neurovirology and molecular biology of degenerative & demyelinating diseases for the past 15 years. He devotes 5% effort to administering this training program.

Dr. Gilden has received numerous outstanding teaching awards by neurology residents, has been elected one of the best doctors in the U.S. by his neurologic colleagues, has received an alumni award for distinguished service by the University of Chicago School of Medicine, has been elected to Fellowship in American Association for the Advancement of Science, elected to the Johns Hopkins Society of Scholars, elected to Honorary Membership in the American Neurological Association, has received the International Society for NeuroVirology Pioneer Award and the Honor Award and Gold Key from the University of Maryland School of Medicine.

Administrative Structure of the Program

All final administrative decisions (program direction, selection of trainees, format for neurovirology-molecular biology lunch seminars, selection of outside speakers, etc.) are made by the senior committee of Drs. Gilden, Tyler and Holmes. In addition, at the weekly neurovirology-molecular biology lunch seminar, discussions and decisions are made by faculty mentors (with input from postdoctoral fellows) regarding the recruitment process and acceptability of candidates, including the acceptance of new mentors, suggestions for scientific seminars, including speakers and topics about ethics in biomedical

research. Essentially, the entire group of faculty mentors, with input from fellows, is advisory to the committee of Drs. Gilden, Tyler and Holmes, a format which has worked successfully since the inception of the training grant.

RESEARCH PROJECTS OF TRAINERS

Jeffrey L. Bennett, MD, PhD

Antigen identification in optic neuritis and neuromyelitis optica

Optic neuritis (ON) is the most common clinically-isolated demyelinating syndrome, and is the presenting feature in many individuals with multiple sclerosis (MS) and neuromyelitis optica (NMO). Dr. Bennett is attempting to identify the primary target of the humoral immune response in these disorders. His laboratory utilizes a RT-PCR protocol to amplify the expressed immunoglobulin variable-region sequences of single B-lymphocytes and plasma cells isolated from ON CSF by fluorescence-activated cell sorting. The B-lymphocyte and plasma cell heavy- and light-chain pairings found in vivo are reconstituted in vitro to produce a panel of recombinant monoclonal antibodies (mAbs) whose specificity is determined by immunocytochemistry, immunoblotting, and screening of white matter and random peptide expression libraries. Since the factors that link ON with subsequent demyelinating disorders remain unclear, projects are ongoing to identify clinical and molecular risk factors that may identify at-risk individuals. The ultimate aim of these studies is to diagnose MS and NMO at the earliest stage of disease and to treat patients with therapies designed to modify or even cure disease.

Mark P. Burgoon, PhD

Antigen identification in chronic neurologic disease

Dr. Burgoon studies the etiology and pathology of multiple sclerosis (MS), as well as the development of techniques to determine the causes of other unknown chronic inflammatory CNS diseases. Dr. Burgoon's laboratory investigates the role of the humoral immune response in CNS disease, particularly those conditions in which intrathecal oligoclonal IgG is present. Currently, his laboratory develops and applies laser capture microdissection and recombinant antibody methodologies to determine the specificities of the immune response and its interaction with the CNS. Dr. Burgoon employs subacute sclerosing panencephalitis, a chronic but fatal measles virus infection of the CNS, as a paradigm to develop these strategies. They are also applying the strategies to the immunoglobulin-producing cells resident in MS lesions. By elucidating the targets of the immune response and how that response develops in the CNS, the laboratory hopes to determine the unknown causes of MS and other inflammatory CNS diseases of unknown etiology, and to develop an effective rationale to prevent disease.

Randall J. Cohrs, PhD

Molecular mechanisms of herpesvirus latency in human ganglia

Dr. Cohrs studies mechanisms of varicella zoster virus (VZV) reactivation in latently infected human ganglia. He has shown that VZV gene 63 is the most abundant and prevalent VZV gene transcribed during latency. A major goal of the lab is to determine the function of VZV gene 63 protein during latency. This is accomplished by characterizing VZV gene 63 protein interaction with other virus and cell proteins using bacterial 2-hybrid systems as well as traditional protein isolation techniques (fast protein liquid chromatography and mass spectrometry). Additionally, the regulation of VZV gene 63 transcription is being investigated. This regulation is studied both at the level of promoter activation and histone modification (epigenetic). Finally, a PCR-based, high-throughput technique in which all VZV

gene transcripts are simultaneously quantitated is being developed to aid in fully characterizing all VZV gene transcripts in latently infected human ganglia. Finally, Dr. Cohrs and his colleagues have shown that subclinical VZV reactivation can result in asymptomatic shed of infectious virus. The Cohrs lab is currently using techniques developed from a long standing collaboration with NASA to determine the clinical significance of asymptomatic virus reactivation and is also using novel bioreactors to propagate VZV in zero-gravity environments.

Donald H. Gilden, MD

Virus latency and gene expression in the nervous system; multiple sclerosis

Dr. Gilden's laboratory has two main thrusts. First is the molecular pathogenesis of varicella-zoster virus (VZV) latency in human ganglia. Studies are designed to determine the cell type(s) containing virus during latency, the configuration of VZV DNA, and the extent of transcription during the latent state. In parallel, varicella latency is being studied in primates with an eye toward developing a model that can be manipulated to determine the mechanism of varicella reactivation. The second large area of investigation includes studies to identify an antigen in multiple sclerosis (MS) brain by analysis of overrepresented IgG mRNA which is then reverse-transcribed into cDNA. These cDNA sequences are then used to prepare recombinant antibodies that are used in antigen identification in MS.

Kathryn V. Holmes, PhD

Molecular pathogenesis of coronavirus infection

Dr. Holmes's laboratory studies the molecular pathogenesis of coronavirus infections and the role of virus receptors in viral pathogenesis. While most coronaviruses infect only respiratory or enteric epithelial cells causing respiratory or enteric diseases, several coronaviruses including mouse hepatitis virus (MHV), feline infectious peritonitis virus and the human respiratory coronaviruses HCV-229E and HCV-OC43 are able to disseminate in vivo and infect other tissues. Some MHV strains are neurotropic in mice and cause acute encephalitis and/or chronic demyelinating encephalomyelitis that have been used to study virus-induced demyelination. Some MHV strains cause persistent infection in vivo, causing relapsing and remitting CNS disease. Other MHV strains cause subacute infection in the CNS with demyelination, followed by clearing the virus infection and active remyelination of the lesions. A recent study showed that a neurotropic and neurovirulent strain of MHV called MHV-JHM is able to infect the brains of mice that lack the MHV receptor, mCEACAM1a. Virus infection spreads rapidly in neurons and astrocytes, and microglia are activated. In contrast, hepatotropic MHV strains cannot infect receptor knock-out mice by any route. The molecular mechanisms for receptor-independent spreading of MHV-JHM infection and glial activation in the brain will be the focus of projects for new trainees.

Gregory P. Owens, PhD

Humoral immunity in multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS in which a complex interaction of environmental and genetic factors confers susceptibility to disease. Although considered autoimmune, the cause of disease is unknown and clues are indicated by increased and persistent intrathecal IgG synthesis. To define the nature of humoral immunity within the CNS, we developed an RT-PCR protocol to amplify the paired heavy (VH) and light (VL) chain variable regions expressed by single CD138+ plasma cells sorted from MS CSF. IgG repertoires generated from CD138+ cells of MS patients display seminal features of a post-germinal center and T-cell dependent antigen-driven response. Memory B cell clonal expansion, somatic hypermutation and the biased use of VH4 gene segments were cardinal features of MS CSF humoral immunity. Whether the primary role of B cells in

MS is through their antibody effector molecules or as antigen-presenting cells, it likely operates through antigen binding to unique and disease-specific surface Ig receptors. Thus the identification of relevant antigenic targets recognized by MS CSF plasma cells is the primary focus of our research. Towards this goal, human IgG 1 recombinant antibodies rAbs derived from MS CSF plasma cell clones will be produced. They will be used to assess the specificity of the intrathecal IgG response and to study mechanistically the role of B cells in disease pathogenesis. Identification of an MS-specific antigen(s) will have wide application, not only for early definitive diagnosis, but for the design of strategies to modulate and possibly prevent disease.

David Patterson, PhD

Mouse models of inborn errors and neurodegenerative diseases

Dr. Patterson's laboratory studies the cognitive disabilities faced by individuals with Down syndrome using mouse models. Current projects include analysis of alterations in brain proteins and metabolism in mouse models of Down syndrome using proteomics and metabolomics approaches. These studies are aimed at developing therapies to alleviate the intellectual and other disabilities seen in Down syndrome. Individuals with Down syndrome have an increased risk of developing Alzheimer's disease at a relatively early age, and the Ts65Dn mouse model of Down syndrome also has brain changes and learning and memory changes that resemble those seen in individuals with Alzheimer's disease, so these studies may illuminate the disease process of Alzheimer's disease as well. Dr. Patterson's laboratory is also creating a mouse model of an untreatable inborn error of metabolism that causes profound developmental delay often accompanied by autistic features in humans. The laboratory has made significant progress in this effort, including identification of new forms of the disorder in humans. These studies include detailed analysis of changes in brain chemistry associated with this condition and studies to understand how these may be reversed.

Jerome Schaack, PhD

Viral transducing vectors

Dr Schaack's laboratory studies: (1) the development and construction of adenovirus gene therapy/transducing vectors and (2) construction and testing of retrovirus vectors. Dr. Schaack directs the Viral Vector Core for the Neurosciences program, and collaborates or interacts with a large number of neurobiologists including Diego Restrepo, PhD, Professor and Director of the Program (neurobiology of olfaction at the systems and cellular levels, neural implants); Kimberly Bjugstad, PhD, Assistant Professor (neural pathway reconstruction and cell replacement in Parkinson's and Down Syndrome); Mark Dell'Acqua, PhD, Assistant Professor (postsynaptic protein kinase and phosphatase signaling complexes in synaptic plasticity); Kim A. Heidenreich, PhD, Professor (molecular mechanisms of insulin and insulin-like growth factor action in the CNS); Manisha Patel, PhD, Associate Professor (oxidative stress in neurodegeneration); Celia D. Sladek, PhD, Professor, Neuroendocrinology (regulation of vasopressin and oxytocin secretion); Alexander Sorkin, PhD, Professor (mechanisms of endocytosis of epidermal growth factor [EGF] receptor); Kenneth Tyler, MD, Professor (molecular and genetic basis of virus-induced cell death [apoptosis] and pathogenesis using the reovirus model); Nancy R. Zahniser, PhD, Professor (regulation of neurotransmitter release, transporters and receptors in brain; mechanisms underlying cocaine and ethanol actions); and W. Michael Zawada, PhD, Assistant Professor (cell replacement therapies in the brain, signaling mechanisms of cell death in Parkinson's disease and effects of alcohol on stem cells).

Kenneth L. Tyler, MD

Virus-induced apoptosis in the nervous system

Dr. Tyler's laboratory uses reoviruses as an experimental model system to study the molecular pathogenesis of CNS viral infections both in primary neuronal cultures as well as in mouse models of encephalitis and myelitis. A major goal of the laboratory is to identify specific cell signaling pathways in infected neurons that play a critical role in mediating virus-induced cell death. Pathways identified in vitro are investigated in vivo in experimental models of encephalitis and myelitis. Pathways that are identified as having a critical role in neuronal death and CNS tissue injury are targeted for manipulation in an effort to develop novel therapeutic strategies for enhancing neuronal survival and reducing virus-induced CNS injury. Recent studies have involved identification of key roles played in cell death by both death-receptor associated and mitochondrially mediated apoptotic signaling pathways, calpain, reactive oxygen species, mitogen activated protein kinases (e.g. JNK), transcription factor regulation (NF-kappaB, c-Jun, TGF-beta), and JAK/STAT signaling.