

# ISNV

*International Society for NeuroVirology*

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Science News  
Page 2

Noteworthy News  
Page 2

NIH News  
Page 3

ISNV Highlights -  
Prasun Datta  
Page 3

ISNV Highlights -  
Susan Morgello  
Page 4

ISNV Highlights -  
Yoshiro Ohara  
Page 5

ISNV Highlights -  
James Lokensgard  
Page 6

ISNV Highlights -  
Samantha Solden  
Page 7

## ISNV Welcomes Avindra Nath as the New ISNV President and Igor Koralnik as Vice President

Dear ISNV Members,

Our goal is to build upon the current strengths of the ISNV and to position the Society to assist clinicians and researchers in not only understanding disease pathogenesis but also developing new diagnostics and therapeutics for infections of the nervous system. To assist with the process, we plan to establish links with the Federal Drug Administration (FDA) and invite speakers from viral diagnostics and viral therapeutics to the ISNV meeting to give us the FDA's perspective on how academic clinicians and researchers can work in partnership with the FDA towards these goals.

Within the last few years there have been a series of outbreaks of CNS infections. The Center for Disease Control and Prevention (CDC) has played a lead role in investigating these outbreaks and in informing the public. At the next ISNV meeting we will invite key researchers from the CDC to give talks on these outbreaks and discuss how the ISNV could work with them to help disseminate the information using their website, journal and newsletters. Since the membership of the ISNV has broad expertise, they could assist in key research questions that need to be addressed and in providing key information to the public in a timely manner.

The ISNV will continue to strengthen its relationship



Avindra Nath, MD,  
ISNV President  
2013-2016

with NIH. A workshop on drug abuse pioneered by NIDA was very successful at the previous meeting in New York. We will try to expand upon it at the upcoming meeting. NIDA might partner with NIAAA for this purpose. NIMH has again expressed interest in possibly conducting a pre-meeting workshop on International aspects of neuro-AIDS.

Another challenge faced by clinicians and researchers is that many of the neurological infections have no diagnostic and therapeutic guidelines. Some viruses can cause multiple clinical syndromes and viruses may also be reactivated in asymptomatic individuals. Hence we would like to develop panels of experts to develop guidelines for these infections and publish them in the Journal of Neurovirology and make them available freely through the website.

Going forward the Society continues to grow but also faces new challenges. Its meetings have been largely funded through grants from the NIH. However, future NIH support for conference grants will be restricted in amounts. Hence alternative means of funding need to be explored. Closer ties will also be developed with other agencies, Societies and pharmaceutical companies that have an interest in neurological infections.

Sincerely,

Avi Nath and Igor Koralnik



Igor Koralnik, MD,  
ISNV Vice-President  
2013-2016

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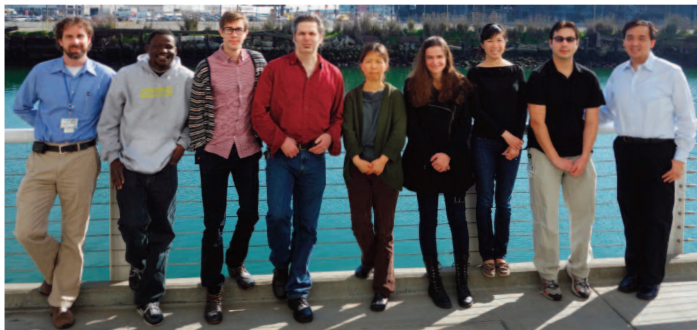
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# Science News

## Novel chip- and sequence-based approaches to identifying viral pathogens

Charles Chui and Fred Krebs



Individuals (from left to right): Steve Miller, MD/PhD; Beniwende Kabre, BS; Jerome Bouquet, PhD; Daniel Wilkerson; Guixia Yu, BS; Samia Naccache, PhD; Deanna Lee, PhD; Erik Samayoa, MS/CLS; Charles Chiu, MD/PhD.

Encephalitis and aseptic meningitis are serious and occasionally life-threatening central nervous system (CNS) infections, with over 75,000 and 19,000 cases, respectively, diagnosed yearly in the United States. For cases where an etiology is identified, the most common infectious agents are viruses (>70% of cases), with the most frequent causes enteroviruses, herpes simplex viruses (types 1 and 2), and arboviruses that are spread through insect vectors. Nevertheless, in most cases, the etiology remains unknown. In the California Encephalitis Project, a 5-year statewide effort to identify causes of acute encephalitis, more than two-thirds of cases were undiagnosed despite extensive testing with a panel of over 30 different assays (Glaser, *et al.*, *Clin Infect Dis.*, 43:1565-77, 2006).

At the University of California, San Francisco (UCSF), new broad-spectrum technologies are being developed to rapidly identify and characterize potential viral pathogens that cause encephalitis / meningitis. Investigators in our laboratory have pioneered the use of the ViroChip microarray, a DNA microarray platform that is able to detect all known as well as novel viruses in a single assay on the basis of sequence homology to highly conserved regions of viral genomes (Chen, *et al.*, *J Vis Exp.*, (50), e2536, 2011). Originally developed in the laboratory of Joseph DeRisi at UCSF, the ViroChip has now been used to detect a number of viruses, including 2009 pandemic influenza A (Greninger, *et al.*, *PLoS ONE.*, 5(10):e13381, 2010), a human cardiovirus (Chiu, *et al.*, *PNAS.*, 105(37):14124-9, 2008), and titi monkey adenovirus (TMAdV), which has been linked to an outbreak of respiratory illness in a captive colony of New World monkeys and a human researcher (Chen, *et al.*, *PLoS Pathog.*, 7(7): e1002155, 2011). Our group has also applied the ViroChip to identify new, divergent enterovirus strains in cerebrospinal fluid (CSF) from

children with aseptic meningitis and encephalitis (Fig. 1).

In parallel with the ViroChip, we are implementing deep sequencing as a complementary tool for virus detection in encephalitis / meningitis. The strength of deep sequencing technology is the ability to detect pathogens at very low titers, such as found in CSF, or that bear little or no sequence homology to any known viral genome. Using unbiased deep sequencing, our team has recently reported the discovery of a new rhabdovirus associated with a hemorrhagic fever outbreak in Africa, provisionally named BASV or Bas-Congo virus (Grard, *et al.*, *PLoS Pathogens.*, 8(9): e1002924, 2012). An Illumina MiSeq deep sequencer has now been placed in the CLIA-certified clinical microbiology laboratory at UCSF, with the capacity to perform a sample-to-answer deep sequencing assay in <48 hours. Through analyses of samples spiked with HIV-1, the limits of detection of this technique for viruses in body fluids such as serum and CSF have been found to be as low as 1-10 viral copies per mL (Samayoa, *et al.*, manuscript in preparation).

Our laboratory is actively engaged in three major efforts related to encephalitis / meningitis diagnosis. First, we are applying these technologies to study unknown cases of encephalitis and meningitis that test negative by all conventional assays done in clinical laboratories. Second, we are investigating CSF and serum samples from a large prospective cohort of acute encephalitis cases in rural India. Third, given the extremely low titers of viruses in CSF in encephalitis / meningitis, we are exploring the use of whole-exome transcriptional profiling by deep sequencing to identify host markers that may assist in differential diagnoses of viral, bacterial, and autoimmune causes and that may be predictive of outcome. The UCSF-Abbott Viral Diagnostics and Discovery Center, under my direction, currently offers ViroChip and deep sequencing analysis of clinical samples in the context of a fee-for-service arrangement or research collaboration (<http://vddc.ucsf.edu>).

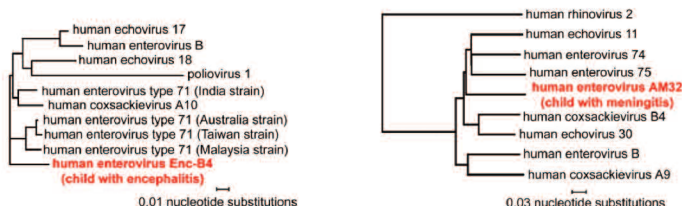


Figure 1. Phylogenetic trees illustrating sequence relationships between known enteroviruses and CSF-derived enterovirus strains (red text) identified using the ViroChip from children with aseptic meningitis and encephalitis.

## Noteworthy News



Dr. Jayasri Das Sarma (pictured right), Associate Professor, Department of Biological Sciences, Indian Institute of Science Education and Research-Kolkata, India and Dr. Randall Cohrs (pictured left), Research Professor, University of Colorado, Denver School of Medicine have received the 2013 ASM-IUSSTF Indo-US Research Professorship Award to develop a bi-lateral research relationship between India and the US. They propose to establish a multiplex array analysis of differentially

expressed host genes involved in innate immunity and demyelination in mice following mouse hepatitis virus infection. Evidence of molecular pathways driving microglial activation in (MHV) infection will advance the understanding of mechanisms by which viral-induced demyelination can occur through innate immune activation. This program is managed by the American Society for Microbiology ([www.asm.org](http://www.asm.org)) and generously supported by the Indo-US Science and Technology Forum ([www.indoustf.org](http://www.indoustf.org)).



# NIH News

## Overview of The Maternal and Pediatric Infectious Disease (MPID) Branch, National Institute of Child Health and Human Development

Rohan Hazara

The Maternal and Pediatric Infectious Disease (MPID) Branch (formerly the Pediatric Adolescent and Maternal AIDS Branch), is in the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The branch supports and conducts a wide range of domestic and international research related to the epidemiology, diagnosis, clinical manifestations, pathogenesis, transmission, treatment, and prevention of HIV infection and its associated infectious (such as tuberculosis, malaria, and hepatitis), as well as non-infectious complications in pregnant and non-pregnant women, infants, children, adolescents, and the family unit as a whole.

Recent initiatives, often in collaboration with NIMH, include diagnostic and pharmacokinetic research in pediatric TB/HIV co-infection, disclosure of HIV status to children in low and middle income countries, perinatally-infected youth in Africa and Asia, and implementation science in prevention of mother to child transmission of HIV.

Our two largest programs are the Adolescent Trials Network (ATN), in collaboration with NIMH and NIDA, and the Pediatric HIV/AIDS Cohort Study (PHACS), in collaboration with 8 other NIH institutes. Both of these programs are domestically based and include extensive neurologic and neurodevelopmental research.

Our neurodevelopmental and neuroscience agenda include the following:

- Domestic and international basic, translational and clinical research in the epidemiology, natural history, pathogenesis,

transmission, treatment, and prevention of HIV infection including neurologic and psychiatric complications in infants, children, adolescents, pregnant women, mothers, women of childbearing age, and the family unit as a whole.

- Neurobiologic and neurodevelopmental effects of human immunodeficiency virus (HIV) infection and other infectious diseases in infants, children, adolescents and pregnant and non-pregnant women.
- Multi-disciplinary studies of the interaction between infectious agents, genetics, brain and behavior including basic science and imaging studies.
- Behavioral interventions to prevent acquisition of HIV and other sexually transmitted infections.
- Effects of drugs to treat HIV and other infectious diseases on neurocognitive outcomes, including the pharmacokinetics/pharmacodynamics interface between central nervous system drug penetration and effects of the drugs and neurologic outcomes and neurotoxicity of drugs used for treatment.
- Neuroscience research on the effects of in utero exposure of the fetus to drugs used to treat HIV and other infections in pregnant women.

More information, including how to contact branch staff, can be found at our website:

<http://www.nichd.nih.gov/about/org/der/branches/mpidb/Pages/overview.aspx>

## ISNV Highlights - Prasad Datta, PhD

Kurt Hauser

At the heart of Dr. Prasad Datta's many current research projects are fundamental questions about transcriptional and epigenetic regulation of inflammatory pathways/effectors in the context of NeuroAIDS and drugs of abuse. Early studies from his laboratory, funded by a Career Development Award from NIH/NIDA, demonstrated that p38 $\alpha$ , MAPK, and MKK6 play prominent roles in IL-1 $\beta$  and C/EBP- $\beta$ -mediated regulation of the complement C3 gene in astrocytes (*J Cell Biochem.*, 112:1168-1175, 2011; *J Neuroimmune Pharmacol.*, 3:43-51, 2008; *Biomed Pharmacother.*, 60:561-568, 2006). These early studies provided a segue into more recent work, for which he has been funded by the NIH to study the role of epigenetics in gene regulation related to HIV infection (*J Cell Physiol.*, 227(7):2832-2841, 2012). Specifically, these studies focus on elucidating the role of epigenetic mechanisms such as chromatin modification and microRNAs in the regulation of the glutamate transporter EAAT2 expression in astrocytes. Results so far demonstrate that HIV-1 induced pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  independently and in combination with morphine, inhibit EAAT2 expression by targeting mechanisms at the transcriptional and post-transcriptional level (Fig. 1, unpublished data). Prasad's long-term goal is to employ pharmacological strategies involving nutraceutical based histone deacetylase (HDAC) inhibitors to upregulate astrocytic EAAT2 expression in the context of NeuroAIDS to mitigate glutamate-mediated excitotoxicity.

In collaboration with Dr. Satish Deshmane (pictured, left), his



Satish Deshmane and Prasad Datta, collaborators on the HIV-1 exosome project.

laboratory is also studying the role of exosomes derived from HIV-1-infected cells on neurodegeneration. Prasad is supported by a pilot grant from the CNAC development core to study the role of exosomes derived from Nef overexpressing cells on neurodegeneration. They have identified unique microRNAs in exosomes derived from HIV-1-infected cells, and also performed proteomic analyses of human fetal neurons treated with exosomes

Continued on page 4

## ISNV Highlights - Prasun Datta, PhD - continued from page 4

isolated from HIV-1 infected cells using a SILAC-based LC-MS/MS approach and have identified numerous host proteins whose expression is dysregulated. These observations demonstrate that exosomes which were considered as “garbage cans” of the cell play a significant role in communication between inflammatory cells and other cells of the CNS, and thus may also have great potential as a vehicle for delivery of therapeutic molecules.

In collaboration with colleagues from the Department of Neuroscience at Temple University School of Medicine, Dr. Datta's laboratory is also investigating potential crosstalk between HIV-1 and glucose metabolism in macrophages and its impact on HIV-1 replication and macrophage survival. Their preliminary studies demonstrate upregulation of pyruvate kinase muscle type 2 (PKM2) and hexokinase (HK) in HIV-1 infected macrophages, activation of HIV-1 LTR by PKM2 in macrophages derived from U937 cells, and increased translocation of HK to outer mitochondrial membrane in activated U1 cells (U937 cell latently infected with HIV-1).

Dr. Prasun Datta received his PhD from the University of Calcutta, India, in Molecular Cytogenetics, in 1990. Prasun joined the Center for Neurovirology while it was part of the Department of Biology in 2005 as a research faculty. He is currently an Assistant Professor in the tenure-track in the Department of Neuroscience,

Temple University School of Medicine, Philadelphia. He enjoys mentoring postdoctoral fellows, graduate and undergraduate students. He is an Academic Editor for *PLoS One* and *Brain Disorders & Therapy*, and reviews manuscripts for numerous scientific journals, including *Journal of Neurovirology*. His laboratory is supported by funds from NIH.

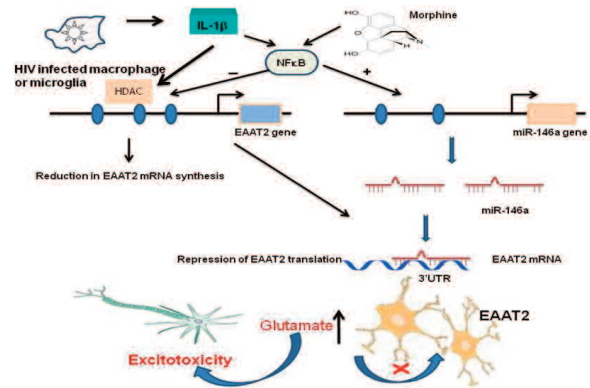


Figure 1. Schematic representation of the mechanism of regulation of EAAT2 by IL-1beta and morphine.

## ISNV Highlights – Susan Morgello, MD

Dianne Langford



Dr. Susan Morgello completed her undergraduate studies at the Massachusetts Institute of Technology, and earned her MD at Duke University Medical School. She completed a residency in Anatomic and Clinical Pathology at The New York Hospital with subspecialty training in Neuropathology. In 1990, she was recruited to the Mount Sinai Medical Center in New York, where she is currently tenured professor of Neurology, Neuroscience and Pathology.

Dr. Morgello is a second-generation neuropathologist, her mother having preceded her in this discipline. Dr. Morgello has benefitted in her career from this unique, multigenerational perspective on disorders of the nervous system. It also helps her to appreciate the extraordinarily rapid development of efficacious therapies for many CNS pathogens that have occurred in the past 2 decades, including those for HIV and other opportunistic organisms seen in patients with AIDS. The shifting phenotype and pathogenesis of HIV-related nervous system disorders have been the focus of Dr. Morgello's research for the past 3 decades.

Dr. Morgello is principal investigator for the Manhattan HIV Brain Bank (MHBB; [www.mhbb.org](http://www.mhbb.org)), a multidisciplinary research

resource she founded with her colleagues in 1998. The MHBB has served to supply well-characterized tissues and fluids for ongoing neuroAIDS research in diverse laboratories, as well as to recruit and train young scientists in the field. The focus of the MHBB is to better define the role of HIV and its co-morbidities in the generation of cognitive and neurologic deficits seen in neuroAIDS. Her group was the first to demonstrate the impacts of literacy on the cognitive assessment of inner city minorities, to show that motoric deficits described in the original American Academy of Neurology nosologies of minor cognitive motor disorders (MCMD) and HIV-associated dementia (HAD) continue to predict HIV-associated neurocognitive disorders (HAND) in the combination anti-retroviral-era (cART), and to demonstrate that peripheral neuropathy is a significant confounder in the neuropsychologic assessment of individuals with HIV. Through attention to careful phenotyping, the MHBB has been able to supply well-annotated tissues that have been the basis for studies demonstrating the impact of cART on global gene expression in brain, the impact of chronic opiate administration on neuroinflammation, and the relationship between expression of ephrin receptors and ligands and HAND.

Dr. Morgello is also principal investigator of the Mount Sinai Institute for NeuroAIDS Disparities (MSINAD; [www.msinad.org](http://www.msinad.org)). This summer institute, conducted by Dr. Morgello along with her colleague Dr. Desiree Byrd, provides mentoring and pilot grant awards to young scientists beginning translational or behavioral neuroAIDS research. The mission of MSINAD is to recruit, educate, and promote the scientific workforce investigating neuroAIDS disorders in minority populations. Thus, the scientific legacy of programs like MHBB will continue into succeeding generations.

## ISNV Highlights - Yoshiro Ohara, MD, PhD

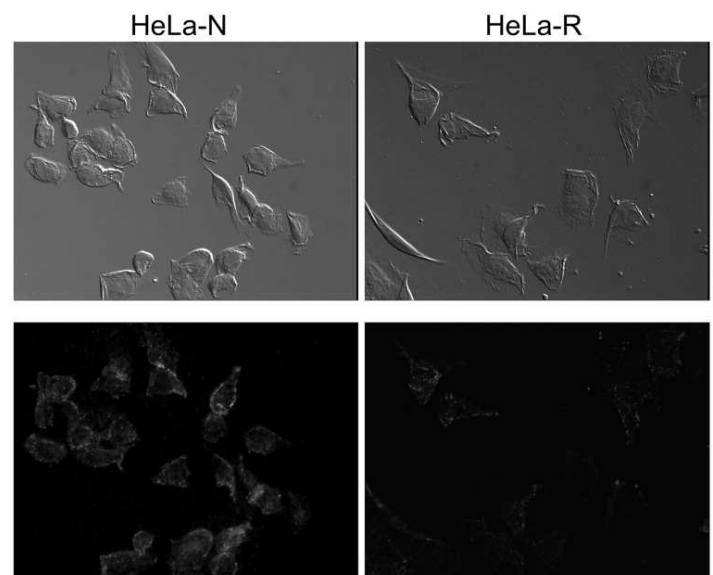
### Dianne Langford

**D**r. Yoshiro Ohara has led a distinguished career as a physician scientist beginning in 1975 when he graduated from Fukushima Medical University with an MD degree. From Fukushima, Dr. Ohara joined Tohoku University and completed his residency training in Neurology and a research fellowship at Yamagata University in the Department of Bacteriology. In 1984, Dr. Ohara earned a PhD from Tohoku University and joined the Department of Neurology at the University of Chicago where he began work on Theiler's murine encephalomyelitis virus (TMEV) under the direction of Raymond Roos. After spending four years at the University of Chicago, Dr. Ohara joined the Faculty in Department of Neurological Sciences at Tohoku University in 1988. In 1994, Dr. Ohara became Chair of the Department of Microbiology at Kanazawa Medical University School of Medicine. After serving as the Dean of Education at Kanazawa for three years, Dr. Ohara then became Dean of the School of Medicine. Currently, Dr. Yoshiro Ohara is Professor and Chair in the Department of Microbiology, Kanazawa Medical University School of Medicine.



As a member of the ISNV for over 15 years, Dr. Ohara's research group has made significant contributions to understanding the pathogenesis of neurotrophic viruses, including Saffold virus (SAFV). Saffold virus is a newly discovered member of the cardiovirus family that was first described in 2007. Since experiments with the murine model indicate that SAFV infects the heart, the CNS and the pancreas, SAFV may be associated with myocarditis, encephalitis, demyelinating diseases and type I diabetes (Himeda and Ohara, *J Virol.*, 86: 1292-1296, 2012). SAFV has been isolated from respiratory and fecal samples of infants with respiratory and gastrointestinal symptoms and from children with non-polio acute flaccid paralysis. In 2011, the Ohara group reported the generation of an infectious cDNA clone of the SAFV-3 (the JPN08-404 strain) that was isolated from cerebrospinal fluid (CSF) of a patient with aseptic meningitis (Himeda *et al.*, *Virology J.*, 8: 110, 2011). The presence of SAFV in CSF of aseptic meningitis patients is potentially important since the closely related TMEV causes a multiple sclerosis-like syndrome in mice. Research on SAFV has led to the identification of 11 genotypes, but the relationship between infection with SAFV and human disease remains elusive. Recent studies from Dr. Ohara's group have

begun to unravel some of the pathogenic mechanisms through which SAFV functions. Using HeLa cells, Himeda and colleagues described lytic and persistent SAFV infection cycles (Fig. 1) (Himeda *et al.*, *PLoS One* 8(1): e53194, 2013). In this study, SAFV-3 was lytic in one subtype of HeLa cells but maintained persistent infection in the other subtype. Unlike TMEV that relies on the interferon response from host macrophages, persistence of SAFV infection in HeLa cells was independent of type I interferon-response. Further analyses suggested that the SAFV persistent infection might be host receptor-mediated. Important implications of these findings in new data from Ohara's group suggest that SAFV-infected inflammatory cells may be migrating into the brain (Ohara, unpublished data). As emerging viruses continue to be discovered, Dr. Ohara's research paves the way for understanding the relationships between infection and human disease.



**Figure 1. Immunofluorescent detection of SAFV binding to the cell surface molecule(s) of each HeLa cell line (arrowheads).** The cells fixed by 10% formalin were incubated with virus (MOI of 100). Then the viruses binding to the cell surface molecule(s) were detected by anti-SAFV-3 antiserum pre-absorbed by the homogenates of HeLa-R cells and Alexa Fluor 594-conjugated anti-rabbit IgG antibody. Left panels: HeLa-N cells, Right panels: HeLa-R cells. Upper and lower panels show Nomarski and fluorescent images, respectively. The viruses binding to cell surface of HeLa-R cells were significantly few, suggesting that the expression of receptor for SAFV infection is low in HeLa-R cells. Magnification: 400x. (reprinted with permission from Himeda *et al.*, *PLoS One*, 8(1): e53194, 2013).

## JOURNAL OF NEUROVIROLOGY



The Neurological Infections Lectureship features prominent investigators who study viral and non-viral pathogens that infect and damage the human peripheral and central nervous systems. Established at the 7th International Symposium on Neurovirology Held in Philadelphia, PA, USA this will be the fifth Neurological Infections Lectureship.

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# ISNV Highlights - James Lokensgard, PhD

Pankaj Seth



Laboratory personnel (left to right): Manohar Mutnal, Wen Sheng, Jim Lokensgard, Shuxian Hu, and Scott Schachtele

Infections of central nervous system (CNS) can lead to devastating consequences ranging from death to neurological sequelae of the infected host. Neuroinflammation following invasion by a pathogen has been identified as a main culprit for injuries to the CNS. Professor James Lokensgard and his colleagues at the University of Minnesota are trying to unravel the mysteries that lead to neuroimmune responses to viral brain infection. Jim and his research team have found that resting microglia (CD11b(+)/CD45(int)) from uninfected brain express very low constitutive levels of MHC class II (<5%), but following murine cytomegalovirus (M)CMV or herpes simplex virus (HSV)-1 brain infection, MHC class II expression is strikingly upregulated on approximately 90% of these cells, including in widespread areas distal to viral infection. Interestingly, this activation within the CNS is not seen following MCMV-infection of IFN- $\gamma$ -knockout animals, yet it can be restored following reconstitution with IFN- $\gamma$ -producing CD8(+) T lymphocytes (Mutnal *et al.*, 2011; Fig. 1). Importantly, these neuroimmune responses persist in the absence of active viral replication and the resident microglia remain chronically activated (>90 d p.i.). Taken together, their findings indicate that resident brain cells react to immune responses generated during viral infection, not simply to the viral proteins themselves.

In more recent studies, Jim and colleagues were surprised to detect numerous CD19(-)CD38(+)/CD138(+) plasma cells and antiviral antibodies persisting within the CNS during chronic herpesvirus brain infection. Findings from his laboratory by Mutnal *et al.*, 2012, demonstrate that while these CNS antibodies are not essential for recovery from acute infection, they do play a significant role in controlling the recovery of reactivated virus. Antibodies produced locally within the CNS most likely have additional effects. For example, in cART-treated, HIV patients with HAND, CNS viral loads are often present below detectable limits, yet chronic microglial activation and its associated neurotoxicity persist. For this reason, it is believed that viral antigen may not alone be responsible. His laboratory is currently investigating whether brain-infiltrating cells of the B lineage and CNS antibodies they produce modulate chronic microglial cell activation through both activating (i.e., Fc $\gamma$ RI, Fc $\gamma$ RIII) as well as inhibitory (i.e., Fc $\gamma$ RIIb) Fc $\gamma$  receptors. He and his research team have also identified a population of CD19(+)/CD1d(high)/CD5(+) regulatory B-cells residing in the brain during chronic infection and are currently studying their

immunomodulatory effects.

Currently, Dr. Lokensgard is applying viral brain infection models to study experimental immune reconstitution disease of the CNS (CNS-IRD) using T-cell repopulation of lymphopenic hosts (TCR $\beta$ -knockout and MAIDS animals) harboring viral brain infection. The goal of these studies is to determine the contribution of brain-infiltrating effector and regulatory T lymphocytes to glial cell hyper-activation and development of CNS-IRD. Their approach is to use adoptive transfer of CD3(+) T-cells into lymphopenic animals followed by assessment of microglial activation. Using Fc $\gamma$ 3-DTR (diphtheria toxin receptor)

expressing transgenic mice they are studying the effect of depleting Tregs from CD3(+) T-cells prior to adoptive transfer into infected, lymphopenic animals. These studies will help determine the mechanisms by which T-cell reconstitution potentiates neurodegeneration.

Dr. James Lokensgard obtained his PhD from the University of Minnesota in 1992, where he now serves as Professor. Dr. Lokensgard has more than 70 publications to his credit with a high citation record. Currently, he serves on several research committees, study sections and as member of the editorial board of the *Journal of Neurovirology*. Jim has mentored numerous students at various levels and has helped the neurovirology community to grow. Dr. Lokensgard's research efforts have been supported by several NIH R01 grants.

Dr. Lokensgard and his research team are optimistic that their research efforts will provide insights into neuroimmunopathogenic mechanisms responsible for CNS-immune reconstitution inflammatory syndrome (IRIS) and translate into development of more effective therapies.

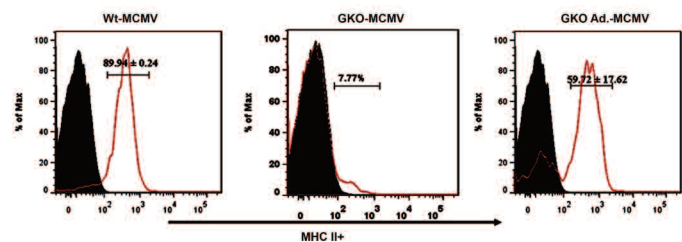


Figure 1. Microglial cell activation is restored following adoptive transfer of wild-type CD8(+) T-cells into IFN- $\gamma$ -knockout (GKO) mice. Histogram overlays using pooled data from infected wild-type (Wt-MCMV), GKO (GKO-MCMV), and GKO mice that received CD8(+) T-cells (GKO Ad.-MCMV) are shown for MHC class II up-regulation on CD45(int)CD11b(+), microglial cells at 30 d p.i.. Grey line (filled) represents isotype control and red line (solid) shows MHC class II expression.

## ISNV Highlights - Samantha Soldan, PhD

Amanda Brown

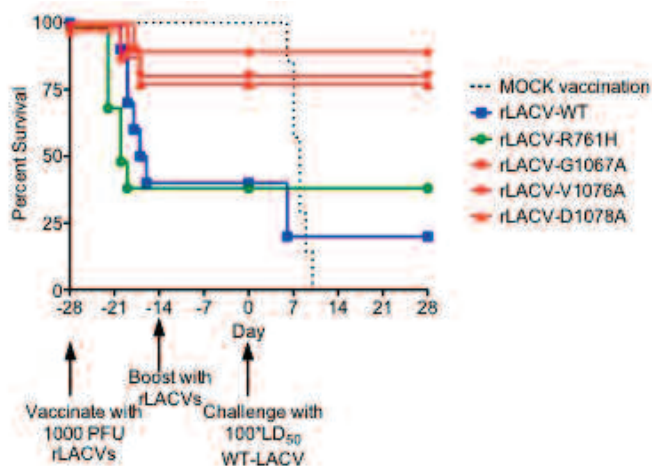
Dr. Samantha Soldan obtained her PhD in molecular genetics through a joint degree program from The George Washington University in Washington DC and the National Institutes of Neurological Diseases and Stroke at the NIH in 2002. In 2003, Dr. Soldan started her postdoctoral training in the laboratory of Dr. Francisco González-Scarano at the University of Pennsylvania; she was subsequently appointed as a Research Assistant Professor in the Department of Neurology at UPENN. In this role, she trains graduate students in neurovirology and directs an independent research group. Dr. Soldan took an early interest in the role of viruses in disease and completed her doctoral studies with Dr. Steven Jacobson on the association of human herpesvirus-6 and multiple sclerosis and participated in studies focused on the immune response to Human T-lymphotropic virus type-1 (HTLV-1) in patients with HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). It was during her postdoctoral fellowship with Dr. González-Scarano that Dr. Soldan began studying the neuropathogenesis of the emerging arbovirus, La Crosse virus (LACV). A leading cause of pediatric encephalitis in the Midwestern, Southern and Southwestern United States, LACV is transmitted by the native mosquito *Orchlerotatus triseriatus* and the invasive mosquito species *Aedes albopictus*. LACV is a member of the Bunyaviridae family, a diverse group of RNA viruses posing significant public health and economic concerns. Importantly, studies of LACV neuropathogenesis serve as a valuable model for CNS studies of neuroinvasion, neurovirulence, and more generally, of arthropod-borne viral encephalitides, including those caused by other bunyaviruses like Rift Valley Fever virus.

The primary objectives of Dr. Soldan's research are to define mechanisms of LACV-mediated neuroinvasion and neurovirulence and to exploit this information to develop vaccines and targeted treatment strategies that are currently unavailable. Dr. Soldan and collaborators previously generated a panel of specific mutations in the fusion peptide of glycoprotein Gc to experimentally confirm the putative location of the fusion peptide based on comparative structural analysis with other class II fusion viral glycoproteins (Plassmeyer, *et al.*, *Virology*, 358(2):273-82, 2007). Recent studies



Individuals (from left to right): Mary-Virginia Salzano, Bradley Hollidge, Samantha Soldan, John Ibrahim, and Jon Fraser.

using a reverse genetics system to generate recombinant viruses containing targeted mutations in the fusion peptide region (Soldan *et al. Virology*, 404(2):139-47, 2010) focused on determining the consequences of mutations in the LACV fusion peptide region on fusion efficiency and virus replication. Currently, the fusion peptide rLACVs are being tested *in vivo* to determine the role of the fusion peptide in neuroinvasion, neurotoxicity, and replication efficiency in the brain and muscle using an age-dependent murine model of LACV encephalitis. In the murine model of LACV encephalitis, newborn mice are sensitive to subcutaneous inoculation of <1 plaque-forming unit of LACV; whereas, weanling and adult mice are resistant to increasingly higher doses administered by the same route. In contrast to peripheral inoculation, intracranial inoculation with LACV is uniformly fatal to mice of all ages, indicating that the virus is neurovirulent even in circumstances where it is not neuroinvasive. Dr. Soldan's group found that in spite of the decreased titers in the brains and spinal cords of adult mice inoculated intracranially with rLACVs with mutations in the fusion peptide region, these mice did not survive longer than those infected with wild-type rLACV, suggesting that the fusion peptide mutant viruses retain wild-type neurotoxicity. However, in suckling mice inoculated subcutaneously, there was a delay in the onset of disease, suggesting that viruses with mutations in the fusion peptide region are less neuroinvasive and slower to enter the CNS. Viral titers for all fusion peptide mutant rLACVs were reduced in the brain, spinal cord, and striated muscle. Of interest, when weanling mice were challenged *i.p.* with fusion peptide domain rLACVs mutants, they had significantly increased survival compared to rLACV-WT inoculated mice (Fig. 1), supporting their hypothesis that the fusion peptide mutant viruses are less neuroinvasive than wild-type LACV. Importantly, additional studies showed that specific prime-boost strategies support the possibility of these mutant rLACVs to serve as possible attenuated vaccines. This finding is exciting because there is relative conservation of the fusion peptide region of disparate bunyaviruses, and therefore, this work could be extended to many other medically significant bunyaviruses and underscores the fusion peptide region as a potential target for vaccine development.



**Figure 1. Fusion peptide domain mutant rLACVs are less neuroinvasive and can protect against subsequent WT-LACV re-challenge.** Weanling mice immunized with 1000 PFU of rLACVs were boosted with 1000 PFU of the same rLACV 14 days following initial rLACV challenge. Subsequently, 14 days following rLACV boosts, the mice were challenged with 100LD<sub>50</sub> of WT-LACV. Mice immunized and boosted with the fusion peptide domain mutant rLACVs were fully protected from lethal WT-LACV re-challenge.

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