

## **The New York Influenza Center of Excellence (NYICE)**

NYICE is a multidisciplinary, multi-institutional, center that emphasizes basic and clinical research on human influenza surveillance, pathogenesis, and host responses to infection and vaccination. Research in the NYICE focuses on clinical studies of influenza infection and vaccination in partnership with immunologists, virologists, and computational biologists. The focus of our center is on the fundamental question of why immune protection from influenza often fails, even in individuals who have generated an apparently robust immune response following infection or vaccination. As part of our surveillance activities, we will collect and analyze the viruses causing influenza infections in vaccinated and unvaccinated subjects and match these to the antibody responses present before infection or at the time illness begins. We will also study the impact of the infections or vaccination on the responding B cell and immunoglobulin (antibody) specificity and function, including development of memory. We will also investigate the nature of T cell help for B cell responses, including antigen presentation and peptide specificity. Optional activities will expand the number of samples available and provide information about the consequences of infection and disease impact, provide additional insight into the role of the innate response in controlling infection and the response to vaccination, and the potential for differences in HA cleavage to account for varying pathogenesis. Overall, these studies address the key question whether vaccine failure results from poor match of the infecting virus and immunity, aspects of the virus, and/or deficiencies in the immune responses to vaccination and infection. The paragraphs below briefly describe the research projects envisioned by NYICE.

### **Antigenic evolution and immunity to influenza**

In spite of substantial efforts to vaccinate, influenza epidemics remain a major public health threat. In the US, the currently licensed vaccines are very safe, but only partially effective at protecting from infection. Though efficacy rates vary with age of the subjects and virus strains, estimates of protection range from 40-70%. As our abilities to study influenza viruses at the molecular level increase, and the costs to do so decrease, there are more reports of a significant degree of sequence diversity in the viruses that circulate seasonally. This raises the possibility that some of the vaccine failures may be due to antigenic drift in the viruses. Unfortunately, there are few studies that have simultaneously assayed the virus sequences, antigenicity, and the specificity or function of the antibodies present in the subject who is infected. As part of our surveillance activities, we will collect and analyze the viruses causing the infections and match these to the antibody responses present before infection or at the time illness begins. We will also study the impact of the infections or vaccination on the responding B cell and immunoglobulin (Ig) specificity and function, including development of memory. These studies address the key question whether vaccine failure results from poor match of the infecting virus and immunity. These studies have the potential to change the way we think about influenza immunity and vaccination, and create a better understanding of immune protection and virus selection.

The overall aims of this project are to test the hypothesis that microevolution and antigenic drift of human influenza viruses contributes to failed antibody-mediated protection by comparing the antigenic characteristics of isolated virus with interrogation of serum and mucosal antibodies, and comparing host immunoglobulin adaptation to HA during infection and vaccination. These deficiencies could arise from antigenic mutations in the virus or through functional defects in the antibodies to the HA. We will also determine whether vaccination and infection drive adaptation of the antibody secreting and memory B cell populations, while performing parallel assessment

of antibody, ASC, and memory B cells over the course of an immune response to infection or seasonal vaccination to determine how these events modify the B cell and antibody repertoires. As a measure of the cells responding to the infection or vaccination, our assays will focus on the acutely activated antibody-secreting plasmablast antibodies. We will also look at in vitro reactivated memory B cells (MBC) and in vitro secreted Ab later in the response as a measure of the adapted responses

### **Targeting B cell responses to provide broad protection against influenza**

With this study we will learn the underlying basis for antibody-mediated immunity to influenza, or a lack thereof, for populations of people at high-risk for infection. We will determine which currently approved or future vaccine composition provides the greatest breadth of protection against all influenza strains infectious to humans, including: Influenza A (H1, H2, H3, H5, and H7) and B strains. We will isolate and characterize a large panel of human monoclonal antibodies that will both inform on protective mechanism and vaccine design and might be directly developed as anti-influenza therapeutics. Finally, we will determine the mechanistic basis for the development of broadly protective antibody responses to influenza both in humans and using transgenic mouse models.

The overall objective of this project is to understand how protective antibody responses to a broad spectrum of influenza strains can be generated, particularly in high-risk human populations such as the aged and very young. The overall project consists of four components, including: Aim 1. To determine what antibody specificities arise in young and aged cohorts that became infected with influenza. With these experiments we hope to learn why people were not protected from influenza infection by antibody-mediated immunity. Aim 2. To compare the efficacy of various vaccine compositions to provide the widest breadth of protection against influenza infection, including the recently emerged highly pathogenic H7N9 strain. Aim 3. To determine the basis of activity for antibodies that have versus those that do not have antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent lysis (CDL) activities. Aim 4. To generate and utilize a transgenic mouse model to explore the mechanistic basis for inducing broadly protective antibodies against conserved influenza epitopes. In the pursuit of these aims, we will also generate a large panel of human monoclonal antibodies that may identify important epitopes that should be targeted for an improved vaccine and also that may be directly developed for use as anti-influenza therapeutics.

### **Links between specificity and function of influenza specific CD4 T cells**

There are several critical voids in our knowledge of influenza specific immunity which is due in part to the complexity of CD4 T cell specificity and function. There is still uncertainty regarding predictors and correlates of protection and a need distinguish the individuals whose immune system is competent to withstand a challenge from those that require boosting or de novo vaccination. We also need to know which vaccines are optimal for eliciting the most effective B cells and T cells. Finally, for pandemic preparedness, it is critical to identify the individuals that can mount a robust response and those whose immune status requires boosts or adjuvant. Our projects will provide the needed new insight into B cell recognition of viral antigen, delineation of the key subsets and antigen specificity of CD4 T cells that convey the needed function and will evaluate newly developed vaccines that may offer significant advantages in elicitation of protective immunity to influenza in human populations.

The core hypothesis that underlies this proposal is that distinct functions of CD4 T cells are carried by cells of different peptide specificities. In some cases, such as help for antibody

responses, linkage of function with specificity may be due to physical coupling of T and B cell epitopes. In other cases, functionality in CD4 T cells may be linked to the context or history of antigen encounter. We will develop the strategies needed to characterize antigen handling/class II processing/presentation by influenza-specific B cells. We will analyze transgenic animals that express the cell surface Ig specific for HA and will characterize antigen recognition, peptide:class II epitopes display and how HA-specific B cells specific for the stalk vs. the head of HA compete for viral antigen acquisition. We will also evaluate the links between immunological memory and potential to respond to seasonal and novel influenza viruses and vaccines. We will determine whether the functional potential of CD4 T cells is correlated with specificity for particular viral proteins, with a focus on provision of help for antibody production, cytokine production, and cytotoxic potential. We will compare immune potential and responses to infection and vaccination in children and adults to determine if maturation of the adapted immune response to influenza potentiates or compromises protective immunity. Finally, there have been new advances in vaccine design for influenza that may offer considerable advantages to protective immunity. We will evaluate the ability of these vaccines to elicit CD4 T cell and B cell responses in human subjects and to better poise individuals for protection from potentially pandemic influenza viruses.

### **Pandemic research plan and response**

The pandemic plan will include both a pre-pandemic risk assessment component, as well as an emergency response plan. The focus of the pre-pandemic risk will be to use age-stratified, banked samples of sera and PBMC, including pre- and post-seasonal vaccine samples, to comprehensively assess the level of population baseline immunity to candidate pandemic threat virus. The emergency response plan will utilize the expertise of our center's assessment of the host response to infection to characterize the host response to an emerging pandemic in humans and relevant animal models, including both the innate and adaptive response to infection and vaccination.

Identifying the levels of immunity pre pandemic can help to guide public health preparedness activities, including predictions of the populations most at risk as well as the expected benefits, if any, of existing seasonal vaccines, and the predicted need for multiple doses of candidate pandemic vaccines. The emergency response plan will help guide strategies to optimize the immunogenicity of vaccination as well as potentially identifying targets for therapeutic agents including repurposing existing drugs that may modify the innate response.

### **Household surveillance -100 sample plan**

The Household Surveillance study will prospectively enroll a random sample of families with young children and perform surveillance for influenza and other respiratory viruses throughout the late fall, winter, and early spring. The specific aims of the household surveillance study will be to define the seasonal occurrence of influenza in Rochester NY, to define the age specific disease burden of non-Medically Attended Acute Respiratory Illness due to influenza, to evaluate risk factors for severe disease, including the effect of vaccination, and to define the dynamics of transmission within households using sequence data, and calculation of the period of transmission and R values in different settings.

Successful completion of this project will allow more precise estimation of the overall disease burden attributable to influenza in a representative population, and the relative contributions of influenza and other respiratory viruses to the overall burden. This information is important in

developing overall public health goals for disease control. The project will also help to resolve controversies related to influenza vaccine effectiveness, and will develop a better understanding of the relationship between influenza genetic variation and transmission within families and populations.

### **Surveillance for Medically Attended Acute Respiratory Illness (MAARI) – 500 sample plan**

The overall aims of Rochester surveillance for MAARI will include defining the seasonal occurrence of influenza in Rochester and the possibility of using social media to enhance surveillance, defining the age specific disease burden of influenza-associated MAARI in comparison to other viruses and the effect of coinfection in all age groups, evaluation of risk factors for severe disease and the effectiveness of vaccination, and to define the role of antigenic variation in the HA and NA in viruses detected in surveillance in escape from immunity in humans

This project will provide important new information about the burden of respiratory disease attributable to influenza in humans of all age groups and the potential impact of coinfection with other respiratory viruses. It will also facilitate studies to characterize influenza isolates from humans and to define the relationship between changes in the antigenic nature of the virus and escape from immunity

### **Optional pathogenesis project: Cleavage activation of influenza viruses**

One of our primary goals in this project is to investigate the cleavage and fusion activation of circulating human influenza viruses with respect to the key proteases acting in the human respiratory tract, and to determine how any modifications to the HA cleavage site impact virus function, pathogenicity or transmissibility. An additional goal of this project is to identify and characterize key proteases (and/or protease activators) from known co-infecting bacteria that can impact HA cleavage and viral pathogenesis. We will characterize circulating influenza viruses with modified HA cleavage sites, identify and characterize proteases from the human respiratory tract that activate influenza HA, and assess the interplay between influenza and co-infecting bacteria for HA cleavage activation

Completion of this project will generate new understanding of the pathologic mechanisms underlying influenza infection of humans and may identify new strategies for influenza control through modifying host or bacterial proteases. In addition, signals that define potentially high threat pandemic candidates will be developed through analysis of the HA protein.

### **Optional Immune Response Project: Systems Biology of Innate Immunity and Vaccination**

The innate immune response to influenza virus is a key determinant of disease severity and subsequent adaptive immunity to infection. As such, modern broad-spectrum vaccination strategies against influenza virus should start with an understanding of the innate immune response. Defining innate immune signaling pathways—and the viral and host factors and interactions that trigger and regulate them—is therefore essential to developing effective drugs and vaccines. In this Project, we will use high-throughput molecular profiling and novel computational methods to build network models of innate immune signaling, identify predictors of vaccine immunogenicity and efficacy, and discover targets and candidate drugs for use as adjuvants or host-directed antiviral therapy. In this project, we will define molecular mechanisms of innate immunity and molecular correlates of robust immune responses and

protection following either seasonal influenza vaccination or passive exposure to seasonal influenza strains. Using computational approaches, we will determine whether existing small molecules or therapeutics can be repurposed for use as adjuvants or broad-spectrum therapies. These studies will be augmented by targeted in vitro approaches to augment our understanding of how influenza virus engages the human innate immune system.

This Project will provide new knowledge regarding the innate immune response to influenza virus infection and vaccination that can be used to develop more effective vaccines (e.g., to elicit a stronger, longer lasting, or more broadly protective immune response). In addition, this Project uses novel computational methods that make use of genomic profiles to rapidly screen small molecules and FDA-approved drugs for repurposing as antiviral therapies. Because many of these compounds have already been evaluated in human subjects, this strategy may significantly reduce the time needed to translate findings into clinical studies.

### **Core clinical support for base projects**

Samples of blood and nasal secretions will be collected from human subjects in three settings: (1) Healthy adults and healthy children ages 6-9, either during the summer (baseline) or following receipt of a licensed vaccine. (2) Adults ages 18-49 receiving licensed egg derived, cell culture derived, or recombinant HA influenza vaccines. Approximately 45 subjects will be studied per year in years 1-4. (3) Subjects of all ages with acute influenza, in support of project 1 (aim1), project 2 (aim 1), and project 3 (aim 2c). Approximately 20 subjects will be enrolled per year. This clinical core will provide samples to host response projects that will define the fine specificity of vaccine induced antibodies and evaluate the relative contributions of follicular T cells specific for the HA or internal viral proteins in providing help for antibody responses. Collaborations welcome! We would be happy to share samples, either to be collected or already in our repository, with investigators at other CEIRS centers.

### **Data Management, Biostatistics and Bioinformatics (DMBB) Core**

The overall objective of the DMBB core is to provide 1) data management expertise and resources at the Center, including LabKey-based data management and repository systems, data integration and quality control, specimen tracking, resource dissemination and data transfer to the CEIRS Data Processing and Coordinating Center (DPCC), 2) provide statistical support in experimental design and data analysis, 3) provide bioinformatics and computational biology support for processing, analyzing and annotating high-throughput bioinformatics data such as RNA-Seq data using state-of-the-art techniques, 4) collaborate and share data and resources with the Models of Infectious Disease Agent Study (MIDAS) program to study influenza transmission and epidemics using the Center's surveillance data, 5) provide expertise in data management, biostatistics, bioinformatics and computational biology to support the Center's education and training program. The DMBB performs a critical Center-wide role providing data management, biostatistical and bioinformatics analytical services/training to address fundamental research questions of why immune protection from influenza often fails, enabling new insights that could lead to more effective control in the face of a pandemic public health threat.

## NYICE people



**John Treanor, M.D.** is the Director of the New York Center of Excellence and leads the clinical core. He received his medical degree in 1979 from the University of Rochester and fellowship in Infectious Diseases at the University of Rochester. Dr. Treanor is currently Professor of Medicine, Microbiology, and Immunology, and Chief of the Infectious Diseases Division at the University of Rochester Medical Center. Dr. Treanor has a long-standing interest in clinical research on influenza and vaccine development, and has been particularly involved in the clinical development of potential live vaccines for pandemic influenza. Dr. Treanor directs the activities of the clinical core, including designing studies to assess the immune response to infection and vaccination, and assessing population immunity to pandemic threat viruses.



**David J. Topham, Ph.D.** is the co-director of NYICE, and leader of project 1, assessing B cell and antibody responses to influenza. Dr. Topham received his Ph.D. in Cell and Molecular Biology from the University of Vermont in 1994, and subsequently trained in the laboratory of Dr. Peter Doherty. He is currently Professor of Microbiology and Immunology, and Vice Provost and Executive Director of the Health Sciences Center for Computational Innovation at the University of Rochester. Dr. Topham is interested in the relationships between host gene expression, the virome, and the microbiome on immune responses and disease outcomes. He will lead project 1, evaluating the specificity and relationship of B cell responses and outcomes of vaccination.



**Andrea J. Sant, Ph.D.** received her Ph.D. in Immunogenetics from Washington University in St. Louis in 1985. She currently Professor of Microbiology and Immunology at the University of Rochester. The research in Dr. Sant's laboratory centers around the molecular events that regulate MHC class II-restricted antigen presentation of influenza proteins by antigen presenting cells (APC) and CD4 T cell recruitment in vivo. Dr. Sant will lead project 3, focused on the CD4 response to infection and vaccination, and the potential impact of CD4 peptide specificity on the development of B cell responses specifically to the HA protein.



**Patrick C. Wilson, Ph.D.** Dr. Wilson received his Ph.D. in Immunology from the University of Texas Southwestern Medical Center in 2000, and post-doctoral training in the Nussenzweig lab at The Rockefeller University in New York City. He currently serves as Associate Professor with tenure in the Department of Medicine/Rheumatology, at the University of Chicago. Dr. Wilson's interests include the development of technology to clone recombinant monoclonal antibodies from discreet populations of B cells. Dr. Wilson will lead Project 2 focused on the B cell response to infection, as well as providing monoclonal antibodies he has, and cloning additional human antibodies specific for influenza HA and NA glycoproteins.



**Hana Golding, Ph.D.** will assist with studies conducted under project 1. Dr. Golding received her Ph.D. in Immunology from Oregon Health Sciences University in 1981, and completed post-graduate work as a Visiting Fellow under Dr. Alfred Singer in 1985 at the Immunology Branch, NCI, NIH, and then as a Visiting Associate in the same branch under Dr. Dinah Singer in 1987. She joined the Food and Drug Administration in 1989, and since 1993 she has served as Chief of the Laboratory of Retrovirus Research in the Division of Viral Products, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA). She will lead the experiments in Aims 1 and 2 of Project 1 to perform Gene Fragment Phage Display Library (GFPDL) studies of antibody specificity, a technique that she pioneered for use in influenza.



**Surender Khurana, Ph.D.**, will assist in the conduct of studies in project 1. Dr. Khurana received his Ph.D. in Biochemistry in 2002 from Delhi University, India. He has been at the Food and Drug Administration since 2002, where he currently serves as Staff Scientist in the Division of Viral Products, Center for Biologics Evaluation and Research (CBEM). His research interests are focused on developing new molecular tools for better understanding of how different vaccine modalities and novel adjuvants improve protective immunity against influenza. Dr. Khurana developed the Surface Plasmon Resonance (SPR) assays which will be used in project 1.



**Masanori Terajima, M.D., Ph.D.** Dr. Terajima received his M.D. in 1988 and his Ph.D. in Developmental Biology in 1994, both from Tohoku University School of Medicine in Japan. Dr. Terajima is a Graduate Faculty Member and Associate Professor of Medicine at the University of Massachusetts Medical School. His research interests include immune responses to influenza virus infection and vaccination, and particularly the development of ADCC and CDL antibody responses. Dr. Terajima will be involved in focusing on molecular and developmental aspects of the CDL and ADCC antibody studies proposed in project 2.



**Michael Katze, Ph.D.** will lead studies to evaluate the innate immune response to influenza vaccine and infection. Dr. Katze is Professor of Microbiology and Associate Director at the Washington National Primate Research Center at the University of Washington. His research focuses on using systems biology approaches to define and model virus-host interactions, innate immune signaling, and the strategies used by viruses to evade cellular defense mechanisms. He has studied influenza virus for over 30 years and has pioneered the use of genomic methods in mouse, ferret, and nonhuman primate models of pandemic and highly pathogenic avian influenza virus infection.



**Gary R. Whittaker, Ph.D.** received his Ph.D. in Microbiology from the University of Leeds in 1991, and completed a postdoctoral fellowship in Cell Biology at Yale University. He is currently Professor in the Department of Microbiology and Immunology at Cornell University. His research interests include the cell biological and biochemical aspects of virus entry, and how virus entry pathways relate to viral pathogenesis. He will assist with sequencing of the influenza virus genomes from the surveillance studies, and help interpret data in the context of HA antigenicity. He will direct experiments to assess the potential impact of bacterial coinfection on the evolution of the influenza HA cleavage site.



**Luis Martínez-Sobrido, Ph.D.** Dr. Martínez-Sobrido received his Ph.D. in Virology and Molecular Biology in 2000 from the Instituto de Salud Carlos III and completed Postdoctoral training at Mount Sinai School of Medicine. He is currently Assistant Professor. He is interested in the use of plasmid-based reverse genetics techniques to rescue recombinant influenza viruses. He will construct recombinant single cycle influenza viruses for assessment of immunity



**Mark Sangster, Ph.D.** received his Ph.D. in Virology/Genetics from the University of Western Australia in 1991. He then completed postdoctoral work in Immunology at St. Jude's Children's Research Hospital. Dr. Sangster is an expert in the analysis of B cell responses to infection and vaccination. His major interests have been mucosal aspects of B cell responses in the respiratory tract and the nature of virus-specific B cell memory. He will perform studies of mucosal antibody and B cell responses, and B cell memory.



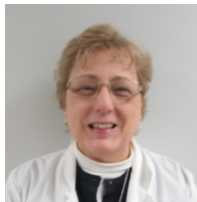
**Peter G. Szilagyi, M.D., M.P.H.** Dr. Szilagyi received his medical degree in 1981 from the University of Rochester and completed his M.P.H. in 1987, also from the University of Rochester. He is a Professor of Pediatrics at the University of Rochester and chief of General Pediatrics. Dr. Szilagyi serves as Director of the Greater Rochester Practice-Based Research Network, a unique network of private practice clinical sites engaged in clinical and translational research. He will serve as the lead investigator of expanded surveillance studies and assessment of disease impact.



**Hulin Wu, Ph.D.** Dr. Wu is Dean's Professor of Biostatistics and Computational Biology and Professor of Medicine (Infectious Diseases Unit) at the University of Rochester Medical Center. Dr. Wu is internationally known for his research in developing novel statistical and bioinformatics methodologies for immunology and infectious diseases. Dr. Wu will be responsible for overseeing and co-directing the operations and activities of the Data Management, Bioinformatics and Biostatistics Core at the Rochester site.



**Jeanne Holden-Wiltse, M.P.H.** Ms. Holden-Wiltse received her MPH from University of Michigan School of Public Health in 1991, and has over 17 years of experience in biostatistical support having held positions in industry, government, not-for-profit organizations and academia. Ms. Holden-Wiltse will act as Co-Leader of the Data Management, Bioinformatics and Biostatistics Core.



**Doreen M. Francis, R.N., C.C.R.C** Ms. Francis received her R.N. in 1973 from the Highland Hospital School of Nursing. She currently serves as Research Administrator and Coordinator for the Vaccine Research Unit in the Infectious Diseases Division at the University of Rochester. Ms. Francis is a highly experienced clinical research coordinator, and she will oversee regulatory and operational aspects of clinical research conducted by NYICE.



**Donna Neu, PMP** Ms. Neu received her B.A. in Computer Science from the State University of New York at Oswego in 1986, and her PMP from the Project Management Institute in 2005. She is currently Project Manager of the New York Influenza Center of Excellence, and the Respiratory Pathogens Research Center at UR. As Project Manager, Ms. Neu will oversee day to day operations of NYICE and coordinate interactions between other CEIRS centers, NYICE and contract operations.