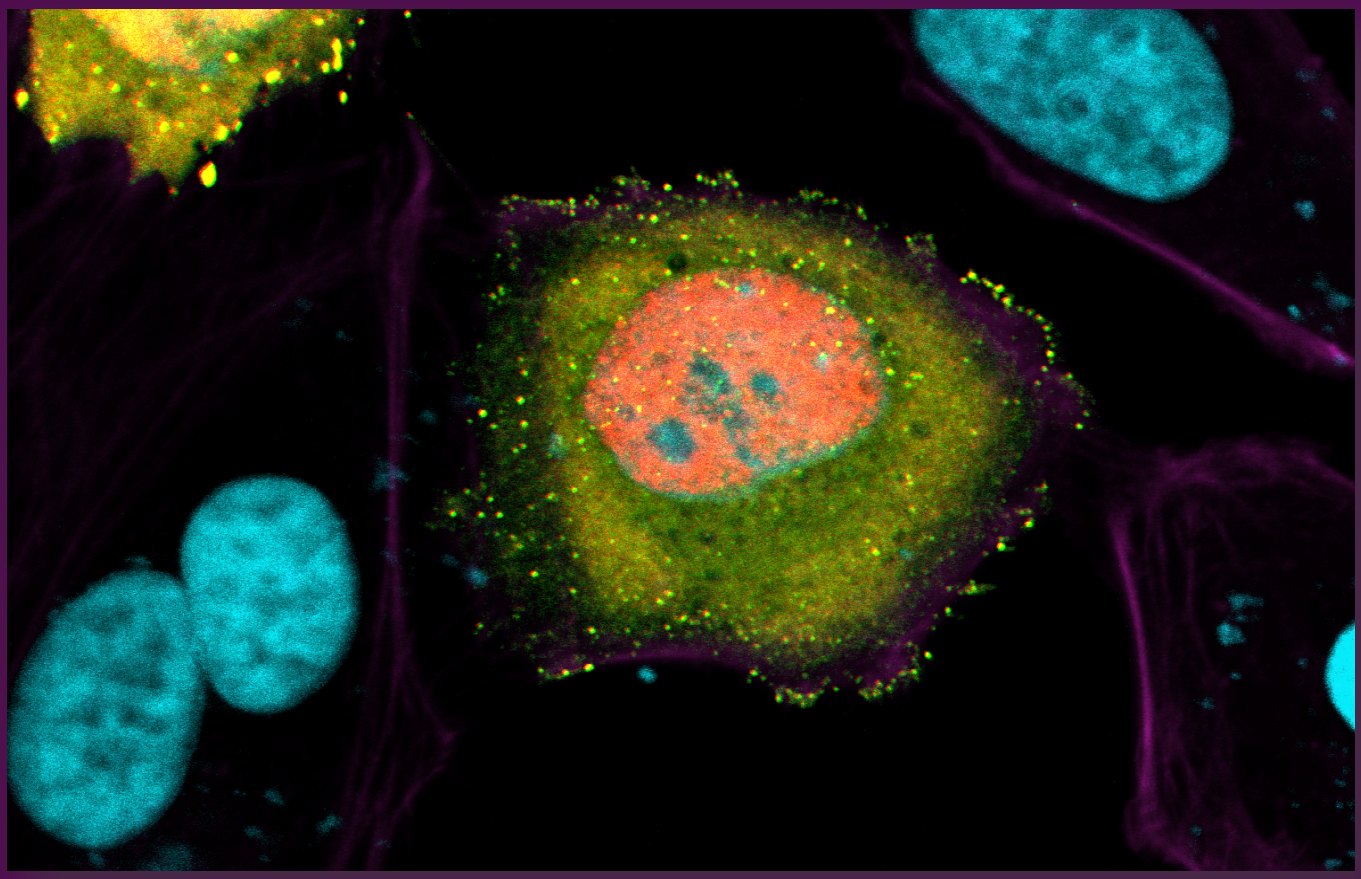

CRIP

Center for Research on Influenza Pathogenesis

Icahn School of Medicine at Mount Sinai

CRIP

The Center for Research on Influenza Pathogenesis (CRIP) is one of five Centers of Excellence for Influenza Research and Surveillance funded by the National Institute of Allergy and Infectious Diseases (NIAID). Influenza viruses are important human pathogens, infecting up to 500 million people annually worldwide, with the most severe pandemic leading to an estimated 40 million fatalities.

CRIP comprises a domestic and international animal influenza virus surveillance network combined with research on pathogenesis and host response. CRIP brings together experts from diverse fields including virology, immunology, molecular biology, veterinary medicine, ornithology, and bioinformatics including several leaders in influenza virus research. The CRIP surveillance network spans all continents, allowing worldwide sampling and isolation of animal and human influenza viruses and early detection of emerging viruses that may cause pandemic threats. Through its research program, scientists at CRIP are dedicated to understanding influenza virus animal reservoirs, evolution, transmission and adaptation to humans as well as pathogenesis, evasion of immunity, and induction of host and vaccine responses.

CRIP also serves as a resource to the scientific community. CRIP provides a repository for viral isolates, serum samples, antibodies, purified proteins, and reagents through BEI, as well as access to viral sequence analysis and phylogenetics through the IRD.

Principal Investigator

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Coordinators

Ryan Camping, Program Coordinator, Icahn School of Medicine at Mount Sinai

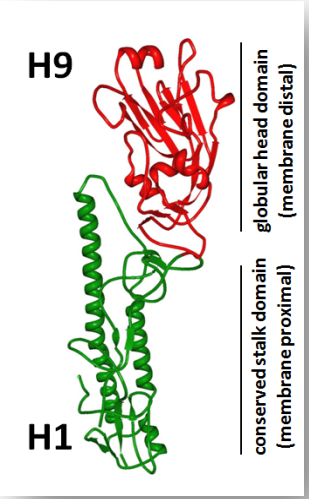
Melissa Uccellini, PhD, Scientific Program Coordinator, Icahn School of Medicine at Mount Sinai

Humoral Responses to Conserved Influenza Virus Proteins

PI: Peter Palese, PhD
Co-I: Florian Krammer, PhD


Classical neutralizing antibodies directed against influenza virus are thought to inhibit the interaction between the major viral surface protein hemagglutinin (HA) and sialylated host cell receptors. These antibodies are directed against the globular head domain of the HA which is subject to antigenic drift, necessitating reformulation of vaccines on an annual basis.

Recently, monoclonal antibodies raised against the conserved stalk domain of HA were found to be broadly protective, and therefore represent the basis for the development of a universal IAV vaccine. Research in project 1 aims to characterize the broadly neutralizing immune response against the stalk domain of HA, as well as conserved domains of the surface protein neuraminidase (NA). The broadly neutralizing antibody response will be characterized in humans as well as animal models in response to both infection and vaccination, with the goal of assessing the feasibility of a universal influenza virus vaccine.



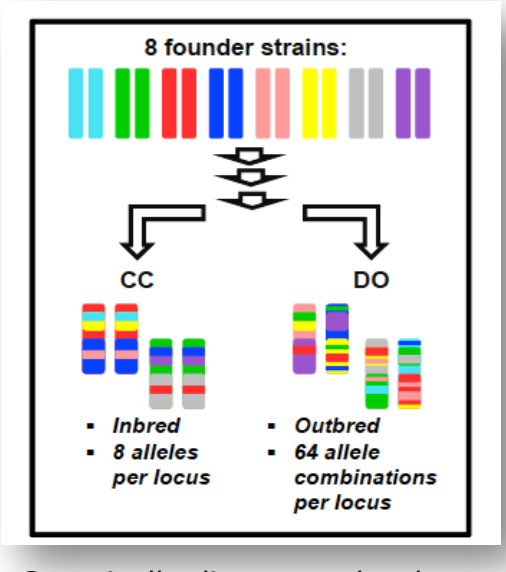
Vaccine constructs containing "exotic" head domains for which humans lack humoral immunity and conserved stalk domains. A series of different head domains and conserved stalk domains are used to sequentially immunize animals in order to boost responses against the stalk domain.

Host Factors and Disease Outcome

PI: Yoshihiro Kawaoka, DVM, PhD
Co-I: Gabriele Neumann, PhD


Despite intensive research, the host factors and mechanisms that determine susceptibility to influenza virus infection remain only partially understood. The Mx1 gene plays an important role in resistance of mice to infection, but other host factors also contribute to pathogenicity. Research in project 2 will use genetically diverse outbred (DO) mouse populations to identify genetic loci which – in – combination with Mx1 – determine the outcome of influenza virus infections in mice. Quantitative trait locus (QTL) analysis will be

carried out by Dr. K. Broman (University of Wisconsin-Madison), an expert in this field. Selected genes that may be critical for influenza virus susceptibility will then be tested experimentally. Further studies will assess the antiviral mechanisms of Mx proteins.

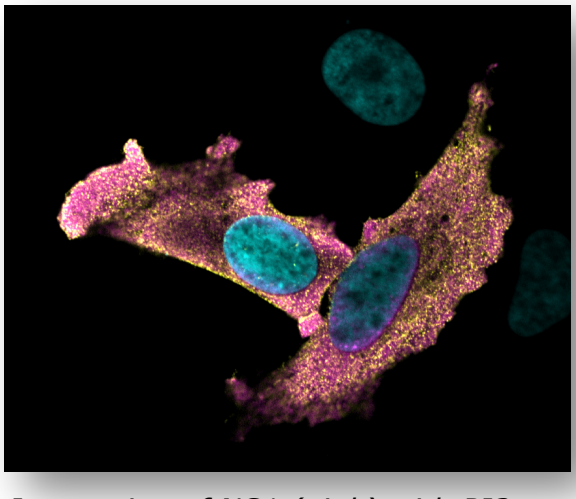


Genetically diverse outbred mouse populations (DO mice) will be used with quantitative trait locus (QTL) analysis to identify loci and genes that are critical for IAV susceptibility.

Host Factors and Virus Tropism

PI: Adolfo García-Sastre, PhD
Co-I: Ana Fernandez-Sesma, PhD
Co-I: Megan Shaw, PhD
Co-I: Randy Albrecht, PhD

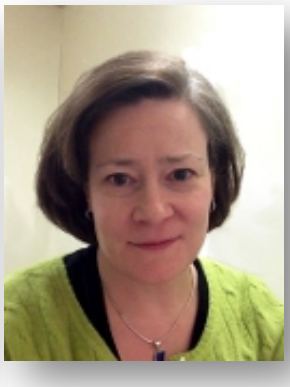
Influenza virus depends on a number of host-derived factors in order to complete its life cycle. We hypothesize that IAV proteins from different host origins such as avian, swine, and human mediate species-specific effects on virus tropism and pathogenesis, resulting in increased or decreased pathogenesis when transmitted between species. Research in project 3 aims to investigate the interactions of various influenza virus proteins – including NS1, HA, NEP and the polymerase complex with cellular host factors. We will also examine how different viral proteins regulate cytokine responses in myeloid cells. Our overall goal is to better understand the impact of virus-host interactions on host tropism and virulence.



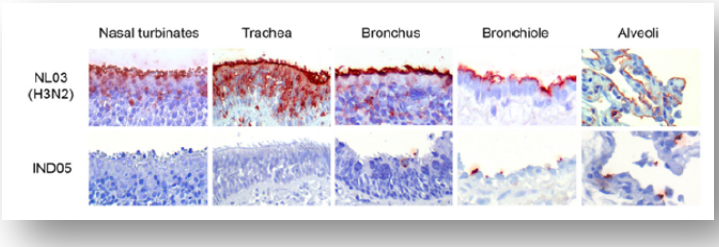
Interaction of NS1 (pink) with PI3 kinase (yellow).

Molecular Determinants of Antigenicity, Virulence, and Transmission of the Influenza Hemagglutinin Protein

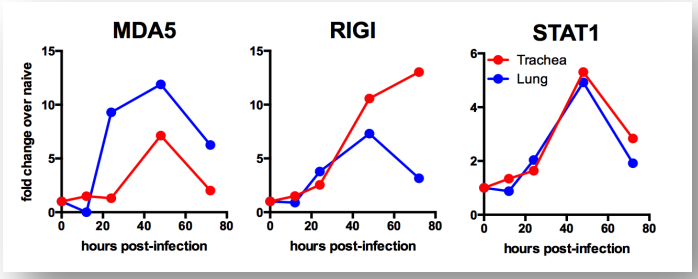
PI: Ron Fouchier, PhD
Co-I: Nicole Bouvier, MD
Co-I: Yoshihiro Kawaoka, DVM, PhD



Influenza viruses are enzootic in wild migratory aquatic birds around the world and occasionally spill over from the avian reservoir into other animal hosts. Introduction of novel influenza viruses from animals to humans, and subsequent reassortment with human strains can result in pandemics. New viruses that emerge in humans may have different virulence and transmission properties, which can largely be attributed to the hemagglutinin protein. Project 4 aims to identify the molecular signatures that determine shifts in antigenicity and increased virulence and transmissibility to provide the basis for a more accurate risk assessment of the pandemic potential of virus strains and the design of countermeasures against virus transmission.



H3N2 and H5N1 viruses show differential binding to human respiratory tissue.

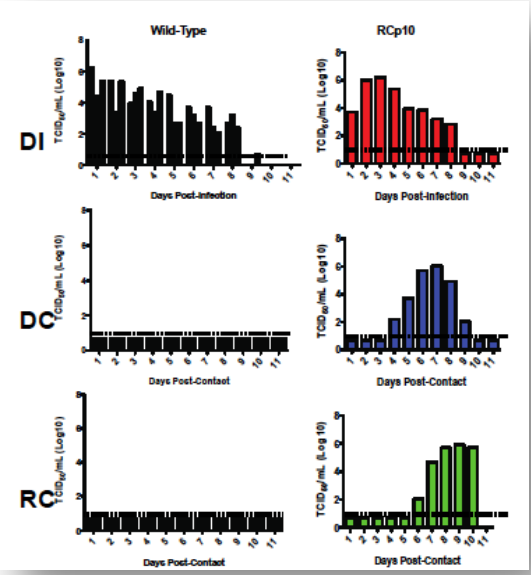


Induction of interferon-stimulated genes in the guinea pig respiratory tract after infection with H3N2 virus.

Transmission of H7 and H9 Influenza Viruses

PI: Daniel Perez, PhD

Infection and transmission of a virus to a new host requires overcoming a number of species barriers including binding to receptors on target cells, blocking host factors that interfere with infection, and interacting with host factors required for replication and release. Emergent viruses must evolve the ability to be readily transmissible to humans by direct and respiratory contact. Project 5 will define the mechanisms that result in respiratory transmission of H9 and H7 influenza viruses in animal models, with the goal of providing more accurate risk assessment of emerging viruses.



WT H7N9 virus does not readily transmit in ferrets. RCp10 respiratory transmissible H7N9 can be transmitted by direct contact (DC) and respiratory contact (RC).

Pathogenicity of Influenza Viruses in Avian Species

PI: David Suarez, DVM, PhD
PI: Erica Spackman, PhD
PI: Mary Pantin-Jackwood, DVM, PhD

The Southeast Poultry Research Center team joined the CRIP Center in 2014 providing expertise in avian influenza virus research and QA/QC for the CEIRS network.

Research

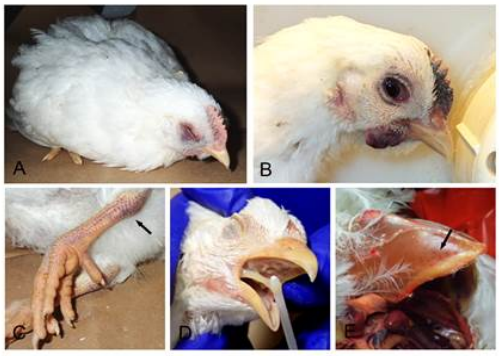
To address pandemic preparedness SERPL evaluates the pathogenicity of novel influenza viruses or viruses with relevant biological characteristics in different avian species including chickens, turkeys, ducks, quail, and other species. Virus-host interactions, including disease presentation, transmissibility and immune responses are examined to better inform influenza virus control.

CEIRS QA/QC Testing

The CEIRS quality assurance and quality control program aims to provide a service to surveillance labs to:

- Assure that virus detection tests are working as expected
- Provide materials that can be used to train and evaluate personnel
- Provide materials that can be used to evaluate new diagnostic tests or modifications to tests

Testing materials include both molecular and serological tests for avian and mammalian origin samples, and are updated frequently to assure recent isolates are included.



Lesions in chickens following experimental infection with the Mexican H7N3 HPAI virus.

CRIP Surveillance Activities

Ron Fouchier, PhD
Yoshihiro Kawaoka, DVM, PhD
Daniel Perez, PhD
Walter Boyce, DVM, PhD
Jonathan Runstadler, DVM, PhD
Rafael Medina, PhD
Gustavo Real-Soldevilla, PhD

The CRIP surveillance team encompasses a domestic and international network for sample collection from a variety of species – including human, avian, swine, and marine mammals – for the rapid identification and characterization of emerging influenza viruses. The goal of surveillance projects is to integrate data to provide for improved risk assessment and to inform mechanisms of viral spread, immunity, virulence and host tropism.

Walter Boyce, DVM, PhD, Jonathan Runstadler, DVM, PhD, Rafael Medina, PhD, and Gustavo Real-Soldevilla, PhD (clockwise from left)

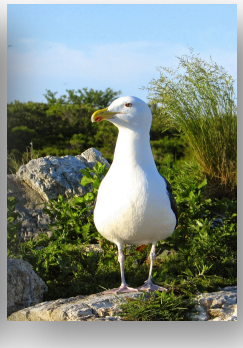
Gull colony in the Netherlands, Iberian pigs in southwest Spain, Pacific harbor seal

Objectives:

- To understand the evolution of influenza viruses in their natural reservoir, upon zoonotic transmission, and during epidemic circulation in mammals
- To understand how host biology and ecology affects virus epidemiology and evolution
- To develop predictive models describing the epidemiology of influenza in wild birds and marine mammals
- To provide skills, expertise, and knowledge to assist during outbreaks and for risk assessment

CRIP Surveillance Sites

Human *– sampling of H5N1 epizootics (Fouchier, Kawaoka), H5N1 in Indonesia and Vietnam (Kawaoka).* ***Avian*** *– sampling of poultry and wild birds in Georgia (Fouchier, Lewis-University of Cambridge), wild birds in Australia (Fouchier, Klaassen-Deakin University), poultry in Indonesia (Kawaoka, Nidom-Ministry of Health), poultry in Vietnam (Kawaoka, Le-National Institute of Hygeine and Epidemiology Vietnam), wild birds in Chile (Medina, Sallaberry), wild birds in Argentina (Perez, Pereda-INTA), wild birds in Guatemala (Perez, UVG), gulls and shorebirds in Alaska (Runstadler, Bishop-Prince William Sound Science Center).* ***Swine*** *– sampling in pigs in Indonesia (Kawaoka, Nidom-Ministry of Health), pigs in Vietnam (Kawaoka, Le-National Institute of Hygeine and Epidemiology Vietnam), pigs in Chile (Medina), iberian pigs and wild boars in Spain (Real-Soldevilla), swine in Argentina (Perez, Pereda-INTA), swine in Guatemala (Perez, UVG), swine in Vietnam (Runstadler, Imani and Ogawa-Obihiro University), swine in Indonesia (Runstadler, Imani and Ogawa-Obihiro University).* ***Marine mammals*** *– sampling northern elephant seals, harbor seals, and California sea lions in San Diego, CA (Boyce, Marine Mammal Center) and in Sausalito, CA (Boyce, Marine Mammal Center), harbor and grey seals in the Gulf of Maine and Northeast (Runstadler, Mystic Aquarium and NOAA).* ***Environment*** *– sampling of potential environmental reservoirs (Runstadler).*



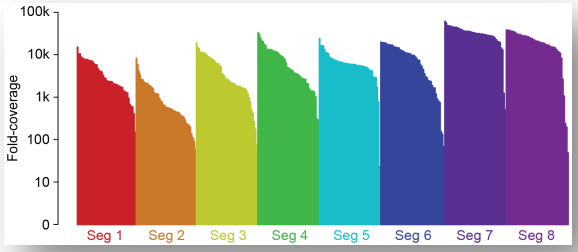
Juvenile Grey Seal in New England, gathering weights on seals, adult Herring Gull.

Influenza Sequencing, Phylogenetics, and Data Management

Harm van Bakel, PhD
Derek Smith, PhD
Eric Bortz, PhD

Sequencing Capabilities

The van Bakel lab at Mount Sinai provides a core resource for sequencing full influenza virus genomes. Optimized multi-segment PCR, automated library preparation, multiplexed Illumina sequencing, and automated assembly provide whole-genome sequences annotated with signature mutations and intra-host variant frequencies. Pac-Bio long-read sequencing allows for phasing long-distance intra-host variants. The Scientific Computing Facility's "Minerva" cluster provides ample computing power and storage for sequencing and analysis.



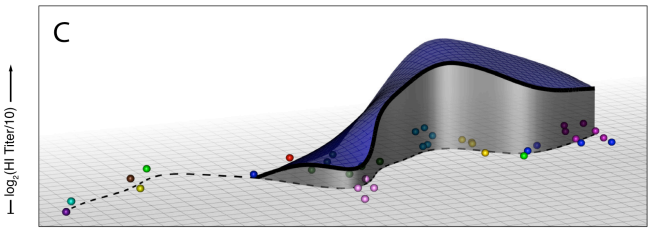
Segment coverage with assembly pipeline

Data Management

The Bortz lab at the University of Alaska Anchorage has established data standards and data identifiers for uniformly cataloging samples including virus isolates, serum samples, and sequences for deposit into public databases such as NCBI Genbank, Influenza Research Database, and BEI Resources. Integrating this data will provide a rich source of information that can be "mined" for current and future projects.

Phylogenetic and Antigenic Analysis

The Smith lab at the University of Cambridge provides expertise in phylogenetic and antigenic analysis of virus isolates with the goal of prioritizing a list of mutations that may increase pathogenicity based on computational and structural prediction. Detailed serological analysis seeks to predict how influenza viruses will evolve in order to inform vaccine design.



Antigenic landscape

Center Coordinators

Ryan Camping
Melissa Uccellini, PhD

Ryan Camping has served as Program Coordinator of CRIP since 2007, ensuring the administrative and financial integrity of all center operations. He is responsible for interacting with the project leaders on all fiscal and administrative issues and serves as a liaison between the members of CRIP and NIAID program staff. In addition, he organizes all aspects of necessary meetings and conferences, prepares semi-annual and annual progress reports, and coordinates the sharing of resources generated by the program.

Melissa Uccellini did her postdoctoral training in the Garc'a-Sastre lab and has recently joined the CRIP team as the Scientific Coordinator. Melissa will be responsible for monitoring the scientific progress of CRIP members, preparing monthly reports, coordinating virus sequencing, and biosafety documentation. In addition, Melissa is involved in research studying influenza pathogenesis in mouse models.

Icahn School of Medicine at Mount Sinai, in New York City