



PROJECT

Supervisor: Nicola Clementi

Title: Molecular Characterization of Respiratory Viruses: From Attachment to Entry Efficiency and Immune Escape Mechanisms

Curriculum: Experimental and Clinical Medicine

Link to the personal page of the University or relevant hospital site website:

<https://www.unisr.it/en/docenti/c/clementi-nicola>

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Viral infections of the upper and lower respiratory tracts are among the most prevalent illnesses in humans, particularly affecting children and infants, who may experience up to five to six episodes per year. As a result, acute respiratory infections (ARIs) continue to pose a significant public health challenge. While most ARIs remain limited to the upper respiratory tract—causing conditions such as rhinosinusitis, pharyngitis, laryngitis, and tracheitis—they can lead to severe complications when spreading to the lower respiratory tract, resulting in bronchitis, bronchiolitis, or pneumonia. The most common acute respiratory viruses are the Human respiratory syncytial virus (RSV), Human Metapneumovirus (HMPV), coronaviruses (including SARS-CoV-2), and influenza viruses.

Since their emergence, respiratory viruses have accumulated mutations that influence infectivity, tissue tropism, and immune evasion. Despite relatively high genetic stability in some cases, key mutations—particularly in surface proteins—can significantly alter transmissibility and pathogenicity. Dominant variants sustaining outbreaks often exhibit distinct profiles in these aspects. This project builds on our laboratory's expertise in virus isolation, whole-genome sequencing, and characterization of viral entry and immune evasion. The primary goal is to establish a general model for monitoring and predicting the properties of emerging variants. To achieve this, we will integrate experimental and computational approaches to analyze past and current variants and apply the same framework to newly emerging ones. Specifically, in vitro assays using various cell lines and inhibitors will assess viral entry efficiency, pathways, and neutralization susceptibility, using both live viruses and pseudoviruses (PVs). Additionally,



computational simulations will evaluate variant-specific receptor affinity and fusion dynamics under different conditions.

Rationale and hypothesis

Viral entry pathways have primarily been studied from the host cell perspective, focusing on entry efficiency in different cell lines or the effects of protease inhibitors. However, the role of viral determinants remains less understood, and a comprehensive picture of how mutations in surface proteins modulate entry is lacking. This project hypothesizes that tissue tropism, immune activation, and antibody neutralization are driven by a dynamic interplay between host cell factors and variant-specific viral protein configurations.

Objectives and specific aims

The project will first define, through in vitro assays, how different variants utilize entry routes, including both cell-free and cell-to-cell transmission. Next, it will assess the impact of viral entry on innate immune activation and antibody neutralization, integrating findings with clinical data to link entry mechanisms with tissue tropism, transmissibility, and pathogenicity. Finally, computational analysis will characterize how specific mutations influence viral entry and immune evasion, aiming to develop predictive tools that reduce the reliance on live virus experiments.

Expected outcomes

This study will provide a detailed molecular understanding of respiratory virus entry and immune interactions at an atomic level. By bridging structural and functional aspects of viral biology, it aims to reduce the need for high-biosafety containment procedures, promoting the use of cost-effective computational modeling for variant characterization and prediction.

Skills that the student should acquire (max. 600 characters including spaces):

The student will develop both experimental and computational skills whose balance can be modulated according to the student and the virus strains that will become available during the PhD as either live virus or PV. The student will be introduced to BSL-2 (for PVs) and BSL-3 (for live virus) working procedures necessary to perform virus isolation and infectivity evaluation. Moreover, the student will become familiar with cell culturing, FACS analysis, confocal microscopy, molecular biology, and sequencing techniques. *In silico* skills will regard the processing of NGS raw data and the molecular dynamics (MD) simulation of the spike protein, either alone or in complex with relevant human proteins..

References (max. 15)



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