

Influenza Virus: Mode of Attack
by
Carmen Polito

College of Medicine Class of 2007
Medical University of South Carolina
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Abstract:

Pathogenesis of influenza virus is of great concern due to its sustained presence in the global community and high rate of morbidity and mortality. This review highlights the mechanism of pathogenicity of the influenza virus and attempts to incorporate recent research findings with regard to lipid rafts in the susceptible host cell membrane.

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Author's Addresses:
polito@musc.edu

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Introduction

The meaning associated with the word "virus" has evolved throughout history much like the evolution of the virus itself. At its inception, "virus" was used to describe any poisonous agent. In fact, venom from a snake was frequently regarded as a sort of virus. Louis Pasteur commonly used the word to describe infections of bacterial origin. In 1898, however, Loeffler and Frosch made formative strides in the field of virology by providing the first evidence of an infectious agent of animals that was not derived from a bacterium [3,11]. At the turn of the 20th century, many bacteria had been shown to cause specific diseases, but scientists were unable to identify a bacterium responsible for causing the highly contagious foot-and mouth disease in cattle, sheep, goat, and pigs [10]. Loeffler and Frosch definitively showed that the infectious agent passed through

a filtering protocol that otherwise removed bacteria; the infectious agent retained its infectious ability even in dilute form. Over a century later, we now define viruses as nucleic acid elements that are able to replicate independently of host cell genetic material but not in absence of a host cell itself [10].

To this end, defining the biochemical mechanism of action and infection of viruses in a host environment has been a monumental research effort in the past century. Influenza virus, like foot-and-mouth disease, is a highly contagious virus belonging to the family *Orthomyxoviridae*. Influenza virus is significant globally in that it is responsible for approximately three pandemics per century and has been documented since 1700 [6]. A pandemic is defined as an outbreak of disease over a large geographic area and a great percentage of the population in that area

[8]. The purpose of this review is to summarize the current research pertaining to the mechanism of action of influenza virus.

Symptoms of infection in the host present themselves 18-72 hours post exposure; this is a fairly short incubation period. The classic symptoms include sudden onset of fever, headache, chills, sore throat, and body aches. Complications of infection may include pneumonia or bacterial superinfections from *Pneumococcus*, *Staphylococcus*, or *Haemophilus*. There are three genera of influenza: A, B, and C. Influenza A strain is responsible for most seasonal outbreaks as well as pandemic and epidemic events. This is due to the mutability of the antigenic glycoprotein, hemagglutinin (HA). The constant evolution of the virus is known as antigenic drift and accounts for why influenza vaccination constituents vary from year to year. Mutations of the viral genome result in the inability of the host immune response to prevent infection; antibodies against other strains of influenza do not bind and therefore inactivate the antigenic hemagglutinin glycoprotein [1,11].

The influenza virus is composed of eight (-) single-stranded RNA helices coated with nucleoprotein; genetic material encodes 10 viral proteins. The virus is enveloped by a spherical lipid bilayer. Interspersed throughout the envelope are the antigenic hemagglutinin (HA) and neuraminidase (NA) glycoproteins. HA is a rod-shaped spike and is responsible for binding to the host cell and fusion of the host and viral membranes. Additionally, hemagglutinin activity results in the agglutination of red blood cells. Agglutination is due to the activity of HA which binds terminal sialic acid sugar residues on both RBC and

respiratory epithelial cell carbohydrates of membrane glycolipids and glycoproteins. The major function of NA is to aid in dissemination of newly formed virus particles via budding. Also present in the lipid membrane of the influenza virus is the M2 ion channel that is responsible for decrease in endosome pH necessary for production of additional HA glycoprotein subunits [1]. Other components of the influenza virus include: matrix protein (M1), nucleoprotein (NP), three polymerases (PB1, PB2, and PA), and two non-structural proteins (NS1 and NS2). (See Figure 1)

Infection of the host occurs when aerosolized particles are transmitted from person to person via coughing, sneezing, or talking. Viral particles enter the respiratory tract of the susceptible host and the HA0 form of hemagglutinin binds to terminal sialic-acid residues on the host columnar epithelial cell membranes in the respiratory tract [1]. Recent research proposes a highly specific mechanism of binding between the virus and host cell. This theory calls into question the fluid mosaic model proposed by Singer and Nicolson in 1972 [5,8]. The fluid mosaic model proposed that membrane proteins exist as icebergs floating in a sea of lipids. However, this new theory of cellular membrane organization characterizes the sea of lipids into what has been termed "lipid rafts." These rafts are subdomains of highly concentrated glycosphingolipids and cholesterol. Highly saturated fatty-acyl side chains and cholesterol allow for tight packing and lateral organization of lipid rafts [5].

The discovery of lipid rafts has been demonstrated by the observation that components of rafts are insoluble in non-

ionic detergents such as Triton-X 100. Consequently, these congregations have been termed DRM (detergent-resistant membrane), TIM (Triton-insoluble membranes), and DIG (detergent-insoluble glycolipid-rich complexes). Separation of these subunits from other insoluble cellular components can be achieved by centrifugation in sucrose gradients [5,7].

Subsequent to their discovery, research has provided evidence that rafts may play a role in cell signaling through several different mechanisms. However, another function of rafts is currently being investigated which involves the "high-jacking" of lipid rafts by exogenous pathogens in an effort to infect a host. Research on the involvement of lipid rafts in the pathogenesis of influenza virus has shown that the glycosylphosphatidylinositol (GPI)-anchored hemagglutinin and neuraminidase proteins are present in sphingolipid/cholesterol raft extractions of cells infected with influenza virus. This evidence suggests that rafts are involved in the mechanism of pathogenicity of influenza virus. More specifically, rafts appear to associate with host epithelial cells at raft congregations in an attempt to gain entry into the cell [7].

Additionally, the same researchers also performed experiments that implicated the actual amino acid sequence of the HA protein transmembrane domain in being responsible for association with lipid rafts - an intrinsic property of the protein. The experimental design involved construction HA mutants containing domains from molecules that have not been shown to associate with lipid rafts. The domains chosen originated from vesicular stomatitis virus

G protein and herpes simplex virus C protein. When inserted into the HA transmembrane domain, HA no longer associated with lipid rafts. This result supports the theory that the amino acid makeup of the HA glycoprotein itself is the determinant in lipid raft association [7].

Concurrent with lipid raft association to HA, other HA spikes on the viral envelope are cleaved by extracellular host proteases into HA1 and HA2 subunits. This cleavage is responsible for the infectious property of the virus. Binding to the host epithelial cell triggers clathrin-dependent endocytosis of the virus and endosome formation. While engulfed in the endosome, the HA2 subunit combines viral and host membranes. The M2 ion channel effectively lowers endosomal pH, resulting in conformational changes in the HA2 subunit. While the C-terminal end of the HA2 glycoprotein remains attached to the virus membrane, the N-terminus effectively fuses the viral and host membranes [1,2,3].

Fusion of both membranes allows the viral ribonucleoprotein complexes (nRNPs) to enter the host cell cytoplasm. nRNP complexes are then transported into the nucleus of the cell where transcription of viral mRNA takes place. Viral polymerases PA, PB1, and PB2 complex and initiate transcription to synthesize a (+)mRNA strand. mRNA is then transported out of the nucleus into the cytoplasm where translation of the mRNA into viral proteins (NS1, NS2, and NP) takes place using host cell ribosomes. These proteins drive a second round of mRNA transcription in the nucleus that synthesizes transcripts whose purpose it is to encode new structural viral proteins. Newly synthesized viral particles re-associate in

the cytoplasm and are disseminated throughout the host by budding [1,2,3,11].

Once the host is infected, major symptoms including fever last approximately 3-4 days. The virus is cleared by successful attack by cytotoxic T cells that kill virus-infected host cells. Antibodies are made to HA and NA glycoproteins and prevent the individual from being re-infected from the same strain. However, due to the high mutability of the HA constituent, antibodies against a particular flu strain does not necessarily safeguard the individual against subsequent exposures to mutated strains. Mutability of the HA constituent is so effective in increasing susceptibility of a population that HA mutations may play the major role in a particular strain reaching pandemic levels [1].

In fact, the Spanish flu outbreak of 1918 was so successful in transmission that 20-30 million people died worldwide. Whereas lethality of influenza usually only results in cases of very young or elderly individuals, this outbreak produced severe illness and high mortality in otherwise young, healthy adults. The causative agent in the Spanish pandemic is thought to be a mutation in the HA and NA subunits and marked the arrival of a new strain of influenza. Additionally, other strains of flu seemed to be less prevalent during this time [1,6,11].

Influenza virus is a complex agent responsible for illness in thousands of individuals each year. The pathogenicity of the virus can be summarized: entrance into the susceptible host in aerosolized particles, HA association with lipid raft constituent of columnar epithelial cells in respiratory tract, fusion of viral and host membranes, transcription and

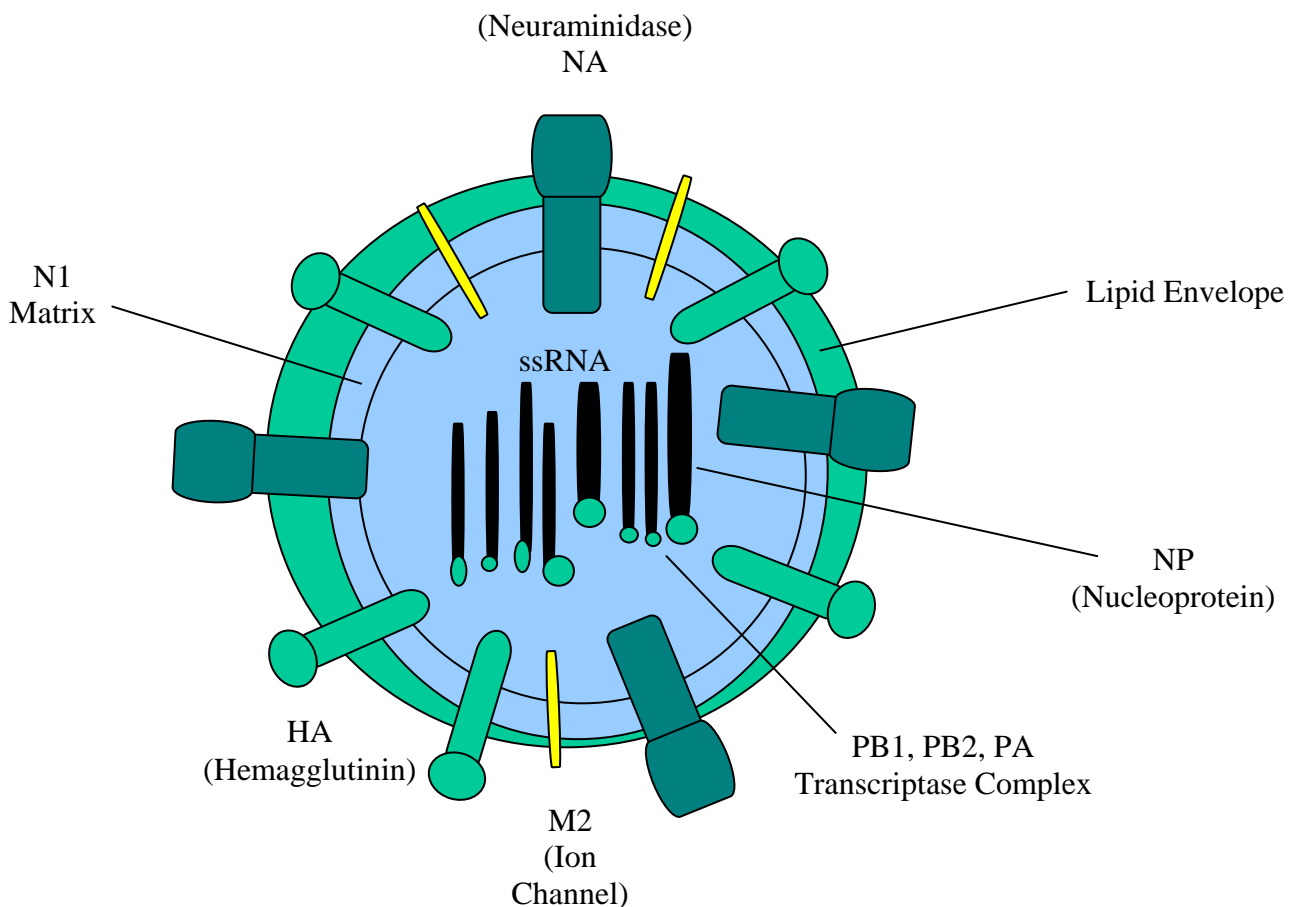
translation of viral genes and protein using host cell machinery, assembly of new viral subunits, and budding of subunits into extracellular host environment. The new involvement of lipid rafts in the mechanism of viral pathogenesis clearly signals the specificity with which viral infection and replication takes place. The success and persistence of influenza virus throughout time is evidence of the efficacy this mechanism provides.

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Figure 1. Structure of Influenza Virus A



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