A Study of the Structure, Organization, Genome, Life cycle, Pathogenicity and Vaccines of the Influenza A virus

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Introduction

Influenza A virus (IAV) is a well-known human respiratory pathogen of the family *Orthomyxoviridae*. Though infection is primarily considered to be limited only to the respiratory system where it can cause very severe pulmonary problems and ultimately can lead to death, extrapulmonary complications such as myocarditis and encephalitis have also been observed. As IAV targets various hosts, including human, swine, avian, equine and poultry, the emergence of novel strains is highly expected, which generates a constant threat of the emergence of unpredictable pandemics.

The occurrence of mutations is another complication in their treatment because it could reduce the resistance to the antiviral therapy or vaccines, making them ineffective. The current vaccines provide only short-term seasonal immunity and need to be administered annually to get maximum result. Therefore it is not surprising that each year more than 1 billion cases of flu, between 3 million to 5 million cases of serious illness and more than 300,000 to 500,000 deaths are reported globally, based on the World Health Organization estimate. Keeping an eye on these problems and the associated numbers, the development of novel and effective anti-influenza strategies is the primary objective.

Influenza A viruses have been isolated from many animal species, but wild waterfowl and other aquatic birds are recognized as their natural hosts. Influenza A viruses detected over the past century have their ancestors in those species mainly. Although many wild bird species may harbor avian influenza viruses, ducks, geese, swans and gulls constitute their primary natural reservoir. Avian influenza viruses are primarily transmitted via the fecal–oral route, which allows the effective transmission between susceptible birds and potentially allows connectivity of virus populations in different host populations.

History

There were 13 severe epidemics during the 18th century and 12 during the 19th century. About 8 of these 25 epidemics were caused by influenza virus. In the 20th century, there have been 4 pandemics (in 1918/19, 1957/58, 1968/69 and 1977) due to the emergence of different subtypes of influenza A virus. The great pandemic of 1918/19 caused an estimated 20 million deaths. Except for 1918/19, the mortality has been predominantly in people of older age groups.

After common cold, influenza or the flu is perhaps the most familiar respiratory infection around the globe. The symptoms of flu are similar to those of the common cold, but they tend to be more severe. Fever, headache, fatigue, muscle-joint pain, sore throat, dry cough, and a runny or choked nose are common symptoms. A number of complications including bronchitis and pneumonia can also occur.

Structure and Organization

The shapes of influenza A virus vary from spherical to filamentous. The spherical forms have diameters of about 100 nm while the filamentous forms can reach as long as 300 nm. The Influenza A virion coat is covered with linear glycoprotein spikes of hemagglutinin (HA) and neuraminidase (NA) which protrude out of the lipid envelope. The ratio of HA to NA is about four to one. Matrix ion channels (M2) pass through the lipid envelope from one end to

another as the transmembrane protein channels. The ratio of M2 to HA molecules is about one to 10^1-10^2. So as a whole, the lipid envelope of influenza A consists of three integral membrane proteins HA, NA, and M2.

Just inside the lipid envelope, a capsid of M1 protein is present which encloses the core and genome of the virus. The nuclear export protein (NEP) is found inside the M1 matrix layer, which is also called non-structural protein 2 (NS2). The ribonucleoprotein (RNP) complex is present in the core, which consists of the viral RNA segments coated with nucleoprotein and RNA-dependent RNA polymerase. The RNA polymerase complex is composed of two "polymerase basic" (PB1 and PB20 and one "polymerase acidic" (PA) subunits.

Genome composition

They are single-stranded negative-sense RNA viruses with a genome consisting of eight segments. Influenza viral RNA (vRNA) has heterotrimeric RNA-dependent RNA polymerase at the 5′ and 3′ end of the segment and the internal part of vRNA is bound with several nucleoproteins (NP) forming viral ribonucleoprotein (vRNP) complexes.

The segments are numbered in order of decreasing length. The segments 1, 3, 4, 5, 6 and 7 encode just one protein per segment: the PB2, PA, HA, NP, NA and M1 proteins respectively. The segment 2 normally encodes for the PB1 protein but in some strains, it also codes for the accessory protein PB1-F2 which is about 87-amino acid long.

The segment 7 encodes for both M1 and M2 matrix proteins. The M2 ion channel is expressed by RNA splicing. The influenza A viruses possess a single RNA segment 8, from which they express the interferon-antagonist NS1 protein and, by mRNA splicing, the NEP/NS2, which is involved in viral RNP export from the host cell nucleus into the cytoplasm.

Segment	Encoded Protein(s)	Protein function
1	PB2 (Polymerase Basic 2)	Polymerase subunit; mRNA cap recognition
2	PB1 (Polymerase Basic 1)	Polymerase subunit; RNA elongation, endonuclease activity
	PB1-F2	Pro-apoptotic activity
3	PA (Polymerase Acid)	Polymerase subunit; protease activity
4	HA (Hemagglutinin)	Surface glycoprotein; major antigen, receptor binding and fusion activities
5	NP (Nucleoprotein)	RNA binding protein; nuclear import regulation
6	NA (Neuraminidase)	Surface glycoprotein; sialidase activity, virus release
7	M1 (Matrix 1)	Matrix protein; vRNP interaction, RNA nuclear export regulation, viral budding
	M2 (Matrix 2)	Ion channel; virus uncoating and assembly
8	NS1 (Non-Structural 1)	Interferon antagonist protein; regulation of host gene expression
	NEP/NS2 (Non-Structural 2)	Nuclear export of RNA

Life Cycle

Life cycle of the influenza A virus can be divided into different stages such as: entry into the host cell, entry of viral RNPs into the nucleus of host cell, transcription and replication of the viral genome inside the nucleus, export of the viral RNPs from the nucleus and assembly and growth at the host cell plasma membrane.

HA is responsible for fusion of the viral membrane with the endosomal membrane after endocytosis, while the NA facilitates the release of virus particles from virus-infected host cells by cleaving sialic acids from the cell surface. The spikes of hemagglutinin (HA) protrude out from the lipid envelope and they bind to sialic acid present on the surface of the plasma membrane of the host cell. The HA1 subunit contains the receptor binding domain and the HA2 subunit contains the fusion peptide. The fusion peptide region is a highly conserved hydrophobic amino acid sequence at the N-terminus of HA2 subunit. This region is important for triggering the fusion reaction and also for destabilizing the cell membrane composition of the target cell.

Two main types of linkages are present between sialic acids and the carbohydrates they get attached to in the glycoproteins. The linkages are α(2,3) and α(2,6), which are very important for the specificity of the HA molecules in binding to sialic acid receptors present in the cell membranes of different host species. Human viruses recognize the α(2,6) linkages, the avian and equine viruses recognize the α(2,3) linkages whereas the swine viruses from can recognize both α(2,6) and α(2,3) linkages. This is the reason that swine is very effective in spreading of both avian and human influenza viruses.

After the binding of HA to the sialic acid receptors on the host cell surface, receptor-mediated endocytosis occurs. Then the virus enters the host cell in an endosome having pH of around 5 to 6. This

low pH causes the fusion of the viral envelope to the endosomal membrane. The fusion peptide at the end of the HA2 inserts itself into the endosomal membrane and completes the fusion. The low pH environment opens up the M2 ion channel, which acts as a proton-selective channel. Opening of the M2 channel makes the viral core acidic and it releases the viral RNP from M1 and the RNP is ready to enter the host cell cytoplasm.

Viral transcription and replication occur inside the nucleus. The protein components of the viral RNP are NP, PA, PB1 and PB2 which can bind to the cellular nuclear import machinery and enter the nucleus. Import of the viral RNP into the nucleus occurs by binding of the proteins to various karyopherins like importin α and β.

The influenza genome consists of negative sense RNA (-ss RNA). Hence before transcription, it must be converted into a positive sense RNA (+ss RNA) to serve as a template for the production of viral RNAs. Replication does not require any primer. The RNA dependent RNA polymerase present in the viral core initiates RNA synthesis. This happens because the 5’ and 3’ ends of the genome exhibit partial inverse complementarity, hence they can form base pair with one another to form different corkscrew configurations. These steps lead to the synthesis of a positive sense complementary RNA (cRNA) and viral messenger RNA (vmRNA) with 5’cap and 3′ poly (A) tail. The influenza virus polymerase does not exhibit capping activity at the 5’end; hence, they have to depend on host-capped mRNAs where they capture its 5’cap through a process known as cap snatching.

The viral m-RNA is translated in the cytoplasm after being exported from the nucleus and viral proteins, and nucleoproteins are synthesized by cellular ribosomes. Translated viral proteins re-enter the nucleus and bind to the vRNAs to generate vRNPs. Following nuclear export, progeny vRNPs and viral proteins are assembled to form virions which later exit the host cell.

Virus Attachment

Influenza viruses recognize N-acetylneuraminic (sialic) acid on the host cell surface. Sialic acids are nine-carbon acidic monosaccharides commonly found at the termini of many glycoconjugates. Thus, they are ubiquitous on many cell types and in many animal species. The carbon-2 of the terminal sialic acid can bind to the carbon-3 or carbon-6 of galactose, forming α(2,3) or α(2,6) linkages; these different linkages result in unique steric configurations of the terminal sialic acid. The sialic acid moiety is recognized and bound by the HA spikes on the surface of influenza viruses.

In human tracheal epithelial cells, α-2,6-linkages predominate, while α-2,3-linkages are more common in duck gut epithelium. Sialic acids with terminal α-2,3-linkages are also present in human respiratory epithelium, though in less abundance than those with α-2,6-linkages. The differential expression of sialic acids in the mammalian respiratory tract may help to explain the low infectivity but high pathogenicity of some avian strains. In humans, α(2,3)-linked sialic acids proteins are most prevalent in the lower respiratory tract (bronchioles and alveoli). The lungs are not as accessible to airborne virus particles as is the upper respiratory tract (nasopharynx, paranasal sinuses, trachea, and bronchi), so avian virus infection is relatively rare in humans. However, when avian strains do infect the human lungs, a severe and rapidly progressive pneumonia may result and fatality rates exceed 60%.

The crystal structure of the HA molecule is a trimer with two structurally distinct regions i.e. a stem, comprising a triple-stranded coiled-coil of alpha-helices, and a globular head of antiparallel beta-sheets present at the top. The head contains the sialic acid receptor binding site, which is surrounded by the predicted variable antigenic determinants. During virus replication, the HA protein is cleaved by serine proteases into two components HA1 and HA2 and this post-translational modification is necessary for virus infectivity.

The HA2 portion is thought to mediate the fusion of virus envelope with cell membranes, while the HA1 portion contains the

receptor binding and antigenic sites. Antibodies to HA neutralize virus infectivity, so virus strains evolve frequent amino acid changes at the antigenic sites. However, the stem-head configuration of the HA molecule remains conserved among strains and subtypes. These relatively minor changes accumulate in a process called antigenic drift. Eventually, mutations in multiple antigenic sites result in a virus strain that is no longer effectively neutralized by host antibodies to the parental virus, and the host becomes susceptible again to productive infection by the drifted strain.

Subtypes and Strains

Influenza A viruses are categorised by the subtype of their surface glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA). The HA and NA receptor-destroying enzymes are different glycoproteins. RNA segment 2, the segment that encodes PB1 also encodes a second polypeptide read in an alternative reading frame, PB1-F2. It varies in length between viruses; the complete protein is about 90 amino acids long, while some strains encode a PB1-F2 of around 55 amino acids and the vast majority of the pandemic A(H1N1) 2009 viruses encode a truncated 11-amino acid long PB1-F2.

Based on antigenicity, 18 subtypes of HA and 11 subtypes of NA are recognized for influenza viruses A. Additional variation occurs within subtypes. In humans, continual evolution of new strains occurs, and older strains apparently disappear from circulation. The majority of neutralizing antibodies are directed to the HA. If NA antibody is present during multicycle replication, it inhibits virus release and reduces virus yield. Antibody to the amino terminus of M2 reduces virus yield in tissue culture. Influenza viruses have a nomenclature system which considers the virus type, the species from which it was isolated (in case of non-human species), place of isolation, isolate number, isolate year and the HA and NA subtype specifically for influenza A viruses.

All the epidemics of respiratory disease in humans since 1900 have been caused by influenza viruses A with the antigenic composition H1N1, H2N2 and H3N2. The pandemics of 1918, 1977 and 2009 were caused by H1N1 viruses, H2N2 caused the "Asian influenza" in 1957 and in 1968 "Hong Kong influenza" was caused by H3N2 virus. H1N2 are reassorted from the viruses H1N1 and H3N2 viruses and they appeared in 2001.

In 1997 and 2003 in Hong Kong H5N1 viruses caused outbreaks in poultry and contemporary illnesses and deaths in humans. H9N2 viruses present in poultry have caused occasional illness in humans in China and were first observed in 1998. Viruses A subtype H7N7 and H3N8 (previously designated equine 1 and equine 2 viruses respectively) cause respiratory disease in horses.

Subtype H1N1 and H3N2 have been isolated frequently from pigs. Swine H1N1 viruses have been found to be reassorted from three different subtypes with their genes coming from swine, avian and human subtypes. Swine H3N2 viruses contain HA and NA genes closely related to those from human epidemic strains. All subtypes of HA and NA, in many different combinations, have been identified in avian species, mainly in wild aquatic birds, chickens, turkeys and ducks.

The structure of the HA, specifically its specificity of binding to the receptor and its cleavability by host protease(s), is important in determining the host range of influenza viruses. The NS1 also contributes to the outcome of infection by mitigating host defence mechanisms through anti-interferon activity.

Interspecies transmission occurs in some instances without genetic reassortment (e.g., the direct transmission of H1N1 virus from swine to humans and vice versa, transmission of H3N2 virus from humans to swine, and transmissions of H5N1 and H9N2 viruses from poultry to humans). In other cases, interspecies transmission may involve RNA segment reassortment in hosts infected with more than one strain of virus, each with distinct host ranges, or epidemic

properties (e.g., 1968 isolates of H3N2 viruses were derived by reassortment between a human H2N2 virus and a virus containing an H3 HA).

Only one species is recognized in the genus Influenzavirus A. The species comprises of a cluster of strains that replicate continuously and can genetically reassort with each other. Total 16 of the 18 HA subtypes of influenza A and 9 of the 11 NA subtypes have been isolated from wild aquatic birds, which serve as a source of novel genes for pandemic influenza viruses. All of them are capable of reassortment of RNA segments.

Avian influenza viruses A are also divided based on their pathogenicity in chickens, into low pathogenic avian influenza A virus (LPAIV) and highly pathogenic avian influenza A virus (HPAIV). Circulation of influenza A viruses of the H5 and H7 subtypes in poultry may result in these viruses becoming highly pathogenic by introduction of basic amino acids in the hemagglutinin cleavage site (HACS). By sequencing of the HACS motif, either a polybasic cleavage site or a monobasic cleavage site can be detected. HA with a monobasic cleavage site can be cleaved by trypsin-like proteases, which are mainly present in the respiratory and gastrointestinal tracts. They affect only the respiratory and gastrointestinal tracts. In contrast, HA with a polybasic cleavage site can be cleaved by proprotein convertases like furin, that are ubiquitously expressed throughout the body. They affect the whole body systematically.

Recently, two new subtypes of influenza were identified in bats (H17N10 and H18N11), neither of these subtypes have been detected in wild birds.

Antigenic Drift and Antigenic Drift

As a result of the antigenic drift, seasonal influenza viruses (SIVs) are generated due to several point mutations in the HA and NA genes caused by RNA polymerase. Thus, the antibodies

generated during primary infection with the influenza virus are unable to neutralize the drifted strains of SIVs, leading to epidemics or pandemics. In addition to SIVs, there are several pandemic viruses generated due to the antigenic shift, where the newly drifted strains of viruses have the ability to cross species barriers, as a result of the re-assortment of a viral genome with other influenza viruses (human or non-human).

Diseases and Transmission

IAVs are categorized as seasonal and pandemic based on genetic variation and the severity of influenza disease. The co-morbidity condition (such as diabetes, heart or liver disease) or the immuno-compromised condition of patients is the predominant cause of mortality associated with influenza virus. Avian IAV such as H5N1 and H9N2 are known to cause bird flu, whereas H1N1 and H3N2 are responsible for swine flu.

IAV transmission occurs by direct or indirect contact, inhaling virus-infected droplets or small droplet nuclei, being exposed to diseased poultry, feeding raw or undercooked poultry, transplacental transmission, or drinking water contaminated by viruses. Influenza virus enters the human body through the respiratory tract and its incubation period is 1–7 days. The common symptoms associated with influenza are respiratory distress, fever, headache, cold, joint pain and abdominal pain.

Patho Physiology

Influenza A virus causes necrosis of the respiratory epithelium in the tracheal and bronchial region and nasal area. Desquamation of the ciliated epithelium, edema, hyperemia, increased secretions, and congestion may therefore result in a secondary bacterial infection. Laboratory findings include leukopenia and often proteinuria.

The influenza A virus can be readily isolated from throat washings following the inoculation of cell cultures or embryonated

chick eggs. During the second week of infection complement-fixing and hemagglutination-inhibiting antibodies are present and can be readily detected. The definitive diagnosis of influenza relies on laboratory procedures like RT-PCR, virus isolation and serological tests. A rapid fluorescent antibody test against the virus is available for direct detection in clinical specimens taken from acutely ill patients.

Vaccines

Influenza vaccines are also called flu shots or flu jabs. New versions of the vaccines are developed twice a year because the virus rapidly changes its composition and structure. The effectiveness of the vaccines varies from year to year but most vaccines provide modest to high protection against the influenza A virus.

As of March 2018, WHO has reported that 46.8% cases are of influenza A virus (where 64% were H1N1 cases and 36% were H3N2 cases) and 53.2% cases are of influenza B virus (where 91% were B-Yamagata strain and 9% were B-Victoria strain).

Two types of vaccines are generally available: the inactivated influenza vaccines (IIV) and the live attenuated influenza vaccines (LAIV). Both types protect against 3 different influenza viruses (hence called trivalent vaccines) such as influenza A(H3N2), pandemic A(H1N1) and 1 out of 2 influenza B lineage viruses. Recent vaccines which protect against 4 different viruses and hence called quadrivalent vaccines, have also been developed. Vaccine should be administered every year to provide maximum protection against influenza infection.

IIV type vaccine is approved for persons 6 months and older including pregnant women and people having chronic medical conditions. One dose is to be injected into the deltoid thigh or muscle. Influenza vaccination given to a pregnant woman will protect both the mother and her newborn baby. Minor side effects can occur including transient fever, muscle pain and other adverse events.

LAIV type vaccine is approved for use only in persons aged 2 to 49 years who do not have any underlying medical conditions. This vaccine is not recommended for pregnant women. It is given as a nasal spray of only 1 dose. It can cause mild symptoms such as nasal congestion, fever or sore throat.

Quadrivalent vaccines contain antigens of influenza A(H1N1) and influenza A(H3N2), as well as two B strains (Victoria and Yamagata lineages). Continuous mutations in influenza viruses can sometimes result in mismatched vaccines. In such cases vaccine effectiveness becomes lower than expected. Effectiveness of a vaccine also depends on the age and health status of the person getting the vaccine, the vaccine product included and the virus subtypes present in the circulation. Vaccination remains the most effective way to prevent influenza infection.

Common side effects include soreness, redness, and swelling where of the spot where shot was given, headache, fever, nausea, muscle pain and fatigue. Signs of serious allergic reactions can include breathing problems, wheezing, paleness, weakness, fast heartbeat or dizziness. These reactions can occur among persons who are allergic to something that is in the vaccine, such as egg protein or other ingredients. There is a small possibility that the vaccine could be associated with Guillain-Barré syndrome, found generally in only1 or 2 cases per 1 million people vaccinated.

Although most influenza vaccines are produced using egg proteins, they are still recommended as safe for people with severe egg allergies. Vaccines produced using other technologies (recombinant vaccines and cell culture based vaccines) have also become available.

Antivirals

Several antiviral drugs inhibiting influenza viruses are available. The most targeted sites for restricting influenza viruses are M2 and

NA, inhibited by antivirals like amantadine, rimantadine, oseltamivir and zanamivir.

Amantadine and rimantadine are called adamantanes. They interfere with viral uncoating but had shown toxic effects that lead to the generation of adamantanes-resistant strains of the virus. Furthermore, progeny virions from host cells is impeded by the neuraminidase inhibitors that caused only one round of replication, hence preventing the spread of infection. Influenza viruses such as A(H3N2) and A(H1N1)pdm09 were observed to be resistant for adamantanes; therefore, for the clinical treatment of influenza virus A, adamantanes are not recommended.

However, influenza A viruses are susceptible to oseltamivir and zanamivir. The other potential targeted sites are viral entry, HA, pH-dependent endosomal fusion, nucleoproteins and polymerases of influenza viruses.

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