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A some new data on influenza viruses

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ABSTRACT. This work represent a synthesis focused on newest data on the influenza virus pathogeny, therapy and vaccinology against H7N9 virus in 2013.
Key words: Influenza virus, H7N9 viruses, viral genome, vaccines

The *Influenza virus* (IFV) particles are highly pleomorphic, but mostly spherical/ovoid, many forms occur. The outer surface of the particle consists of a lipid envelope from which project prominent glycoprotein spikes.The inner side of the envelope is lined by the matrix protein.The genome segments are packaged into the core. (Fig.1)

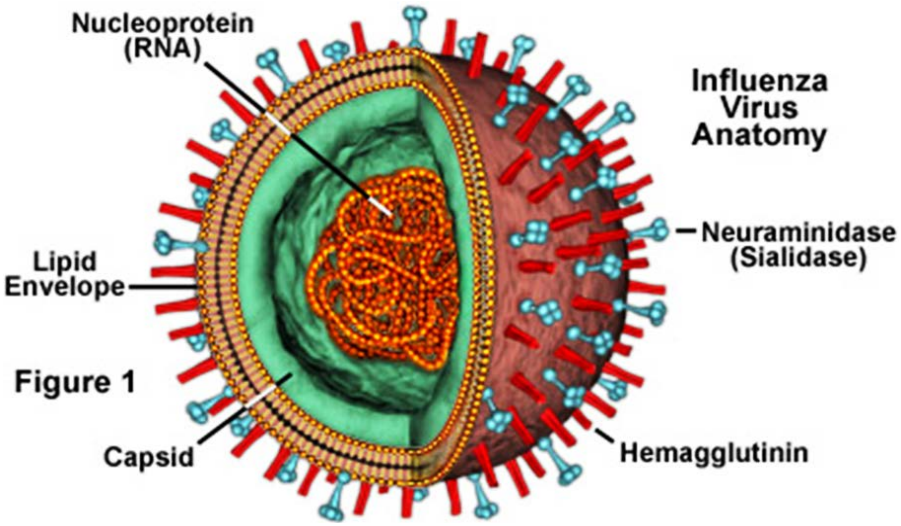

Fig. 1. In the lipid bilayer there are two integral membrane proteins: Hemagglutinin "H" and neuraminidase "N"

Taxonomy
Family Orthomyxoviridae:
1.Genus Influenzavirus A
Type species influenza A virus
2.Genus Influenzavirus B
Type species influenza B virus
3.*Genus Influenzavirus C*
Type species influenza C virus
4.*Genus Thogotovirus*
Type species Thogoto virus
Types of influenza virus
There are three types of influenza:
Influenza A – Is responsible for regular outbreaks, including the one of 1918. Influenza A viruses also infect domestic animals (pigs, horses, chickens, ducks) and some wild birds.

Influenza B - Often causes sporadic outbreaks of illness, especially in residential communities like nursing homes. Influenza C - Common but seldom causes disease symptoms
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The genomic organization of influenza A virus
The (-) strand RNA genome comprises 8 segments, each encodes at least one protein:
1) 3 distinct hemagglutinins: H1, H2, and H3
2) 2 different neuraminidases N1 and N2
3) nucleoprotein
4) matrix proteins

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5) NS (nonstructural proteins, that are not incorporated into viral particles) gene encodes two different non-structural proteins (6-8) subunits of RNA polymerase

Tab. 1. The functions of viral influenza genomic proteins

Segment*	Protein	Function
1	PB2	Polymerase component
2	PB1	Polymerase component
3	PA	Polymerase component
4	HA	Hemagglutinin, viral attachment protein, fusion protein, target of neutralizing antibody
5	NP	Nucleocapsid
6	NA	Neuraminidase (cleaves sialic acid and promotes virus release)
7†	M_1	Matrix protein: Viral structural protein (interacts with nucleocapsid and envelope, promotes assembly)
	M_2	Membrane protein (forms membrane channel and target for amantadine, facilitates uncoating and HA production)
8†	NS_1	Nonstructural protein (inhibits cellular messenger RNA translation)
	NS_2	Nonstructural protein (important but unknown function)

*Listed in decreasing order of size.
†Encodes two messenger RNAs.

Subtypes of influenza A The hemagglutinin of the 1918 flu virus was H1, its neuraminidase was N1, so it is designated as an H1N1 "subtype". Flu pandemics occur when the virus acquires a new hemagglutinin and/or neuraminidase.

Influenza A Viruses

Infect a wide variety of mammals, including man, horses, pigs, ferrets and birds. Pigs and birds are believed to be particularly important reservoirs, generating pools of genetically/antigenically diverse viruses which get transferred back to the human population via reassortment (close contact between pigs and man in the far east; Ducks - migration!).

The main human pathogen, associated with epidemics and pandemics.

Influenza A Viruses. The morphology

Virions enveloped with about 500 spikes.His morphological structure include:

1. Nucleocapsid enclosed within lipoprotein membrane
2. Virions contain 8 segments of linear negative-sense single stranded RNA
3. Total genome length is 13588 nt
4. The largest segment 2341 nt(nucleotide)

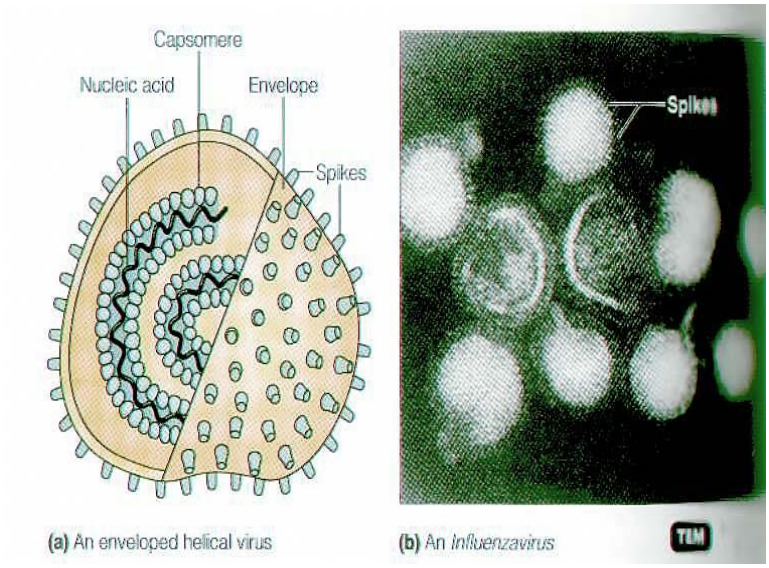
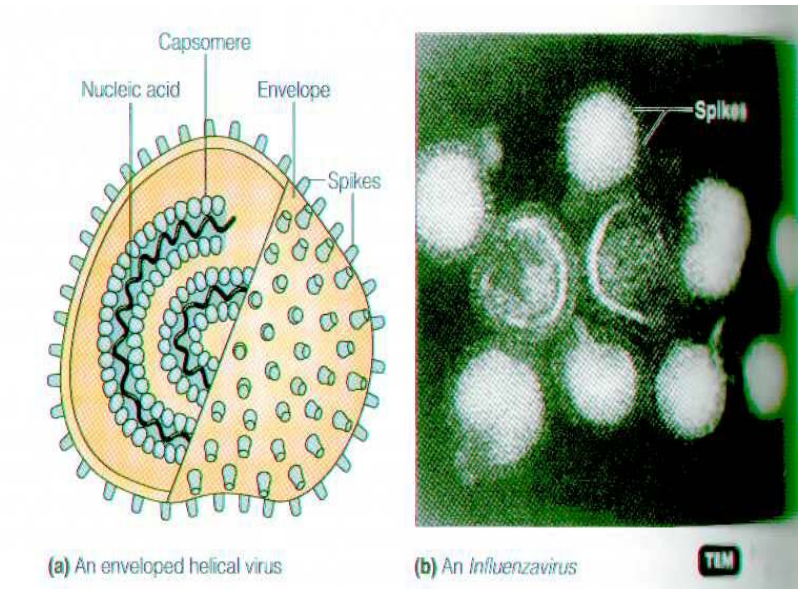


Fig.2. Influenza virus B. The morphological characteristics:

1. Virions enveloped with about 500 spikes
2. Nucleocapsid enclosed within lipoprotein membrane
3. Virions contain 8 segments of linear negative-sense single stranded RNA
4. Total genome length is 13588 nt. The largest segment 2341 nt

Influenza virus C. The morphological characteristics (Fig.3):

1. Virions enveloped, with many spikes
2. Nucleocapsid enclosed within lipoprotein membrane
3. Virions contain 7 segments of linear negative-sense single stranded RNA
4. Total genome length is 12900 nt.The largest segment 2300-2500 nt



Influenza C viruses infect man alone, but do not cause disease (?).
They are genetically and morphologically distinct from A and B types - little studied.

<u>The mechanisms of infection</u>

The influenza virus have a single-cell reproductive cycle with the several steps:
1.Attachment to the epithalial cells of the host through hemagglutinin.
2. Endocytosis
3. Uncoating -> This exposes the contents of the virus to the cytosol.

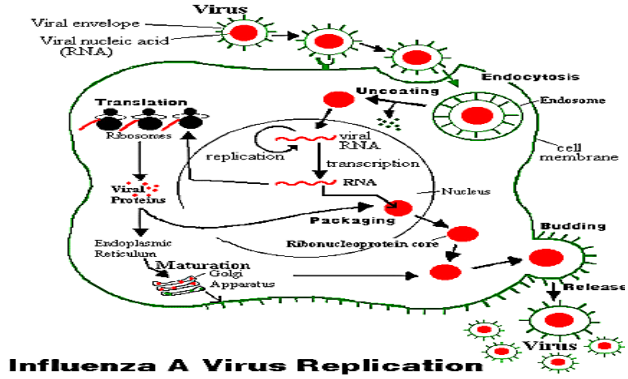
4.The RNA enter the nucleus of the cell where fresh copies are made.
5. These copies return to the cytosol where some serve as mRNA molecules to be translated into the proteins of fresh virus particles.
6. Fresh virus buds off from the plasma membrane of the cell (aided by the neuraminidase) thus spreading the infection to new cells.

The viral Assembly, little is known:
1. Packaging
2. NS2 binding and export of the nucleocapsid to the cytoplasm
3. M1 directs the nucleocapsid to the membrane
4.Viral proteins reach the site
5. Budding
6. Release

The viral Replication

The virus attaches to the outside of the host cell and its RNA enters into the cell. The viral genes are transcribed and translated by the cell's enzymes and ribosomes. In this way, the virus takes over the cell's productivity.

Fig.4. Influenza AVirus replication



The life-cycle of IFV

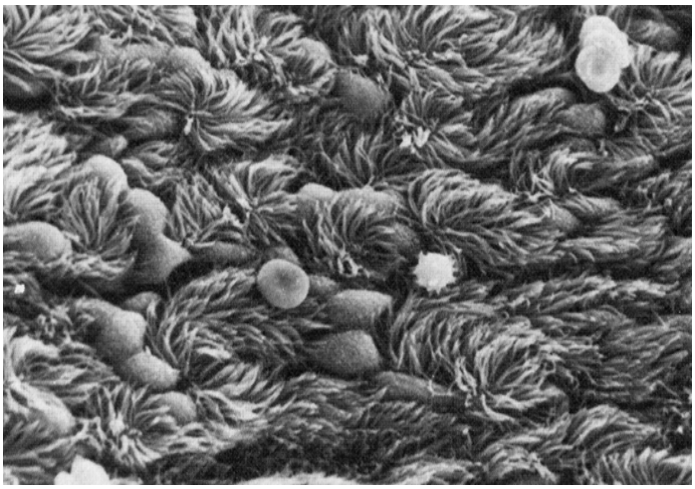
- New viral proteins are translated from transcribed messenger RNA (mRNA).
- New viral RNA is encased in the capsid protein, and together with new matrix protein is then transported to sites at the cell surface where envelope haemagglutinin and neuraminadase components have been incorporated into the cell membrane.
- Progeny virions are formed and released by budding.
- The cell does not die (at least not initially).
-

Pathogenesis of IFV infection

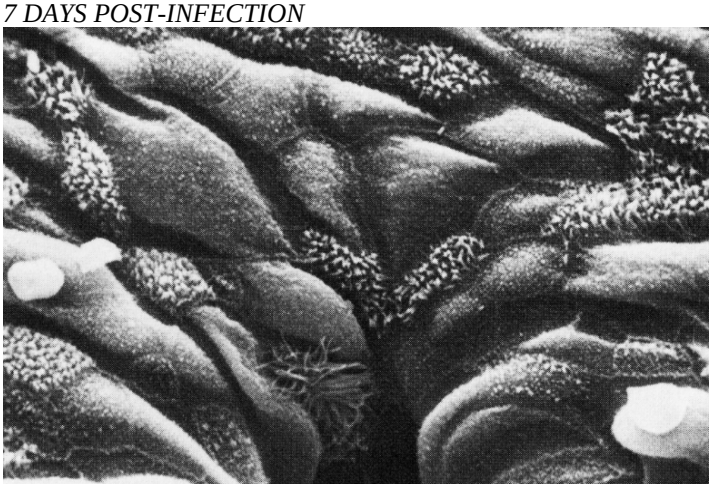
The virus is spread by aerosols. The influenza virus invades cells of the respiratory passages. Primary infection involves the ciliated epithelial cells in the nose, throat and intestines of birds or humans. Necrosis of these cells results in the usual symptoms of the acute respiratory infection (fever, chills, muscular aching, headache, prostration, anorexia).Normally self-limited infection usually lasts 3-7 days.
It usually does not kill the patient (the 1918 pandemic was an exception; some victims died within hours) but does expose the lungs to infection by various bacterial invaders that can be lethal. Damage to respiratory epithelial cells predisposes to secondary bacterial infections which accounts for most deaths.

Fig. 5. The impact on tracheal mucosa

NORMAL TRACHEAL MUCOSA



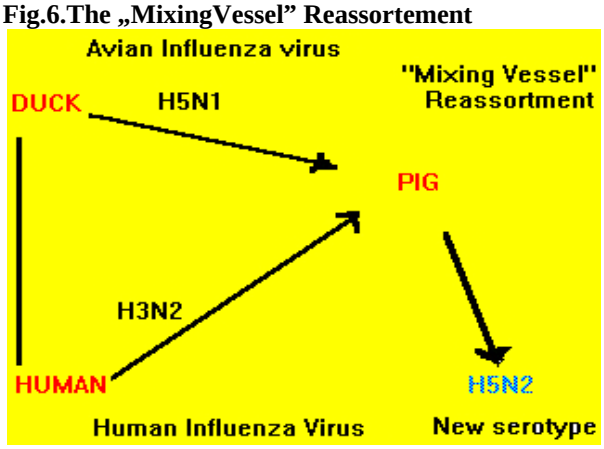
7 DAYS POST-INFECTION



Virus reassortment

The Haemaglutinine and The Neuraminidase are encoded by separate RNA molecules.
If an animal is simultaneously infected by two different subtypes, these genes can be reassorted. For example: pigs simultaneously infected with swine flu virus (H1N1) and the Hong Kong virus (H3N2): H3 and N1 are reassorted in a pig and a new H3N1 virus appears. Reassortment can also occur in humans with dual infections.

Fig.6.The „MixingVessel" Reassortement



Which viruses cause highly pathogenic disease?

Influenza A viruses have 16 H-subtypes and 9 N-subtypes. Only viruses of the H5 and H7 subtypes are known to cause the highly pathogenic form of the disease. However, not all viruses of the H5 and H7 subtypes are highly pathogenic and not all will cause severe disease in poultry. On present understanding, H5 and H7 viruses are introduced to poultry flocks in their low pathogenic form. When allowed to circulate in poultry populations, the viruses can mutate, usually within a few months, into the highly pathogenic form. This is why the presence of an H5 or H7 virus in poultry is always cause for concern, even when the initial signs of infection are mild. ***Recently, a novel subtype influenza A (H7N9 seems to be an other pathogenic agent.***

Clinical features

Influenza is characterized by fever, myalgia, headache and pharyngitis. In addition there may be cough and in severe cases, prostration. There is usually not coryza (runny nose), which characterizes common cold infections. Infection may be very mild, even asymptomatic, moderate or very severe.

What are the implications for human health?

1. The widespread persistence of H5N1 in poultry populations poses two main risks for human health.

The first is the risk of direct infection when the virus passes from poultry to humans, resulting in very severe diseases.

Of the few avian influenza viruses that have crossed the species barrier to infect humans, H5N1 has caused the largest number of cases of severe disease and death in humans.

Unlike normal seasonal influenza, where infection causes only mild respiratory symptoms in most people, the disease caused by H5N1 follows an unusually aggressive clinical course, with rapid deterioration and high fatality.

Primary viral pneumonia and multi-organ failure are common. In the present outbreak, more than half of those infected with the virus have died. Most cases have occurred in previously healthy children and young adults.

A second risk, of even greater concern, is that the virus – if given enough opportunities – will change into a form that is highly infectious for humans and spreads easily from person to person. Such a change could mark the start of a global outbreak (a pandemic).

What about the pandemic risk?

A pandemic can start when three conditions have been met: a new influenza virus subtype emerges; it infects humans, causing serious illness; and it spreads easily and sustainably among humans. The H5N1 virus amply meets the first two conditions: it is a new virus for humans (H5N1 viruses have never circulated widely among people), and it has infected more than 100 humans, killing over half of them. No one will have immunity should an H5N1-like pandemic virus emerge.

And now, a new influenza virus:

Severe disease in humans caused by a novel influenza A virus that is distinct from circulating human influenza A viruses is a seminal event. Therefore, the discovery of novel influenza A (H7N9) virus infections in three critically ill patients recently reported in the Chinese Journal

by Gao and al. An novel reassortant avian-origin influenza A (H7N9) virus was isolated from respiratory specimens obtained from all three patients and was identified as H7N9. Sequencing analyses revealed that all the genes from these three viruses were of avian origin.

A new alert

A novel reassortant avian-origin influenza A (H7N9) virus was isolated from respira-

tory specimens obtained from all three patients and was identified as H7N9. Sequencing analyses revealed that all the genes from these three viruses were of avian origin,

with six internal genes from avian influenza A (H9N2) viruses.

Substitution Q226L(H3 numbering) at the 210-loop in the hemagglutinin (HA) gene was found in the A/Anhui/1/2013 and A/Shanghai/2/2013 virus but not in the A/Shanghai/1/2013 virus. A T160A mutation was identified at t he 150-loop in the HA gene of all three viruses.

A deletion of five amino acids in the neuraminidase (NA) stalk region was found.

All three patients presented with fever, cough, and dyspnea.Two of the patients had a history of recent exposure to poultry. Chest radiography reveale fuse opacities and consolidation. Complications included

acute respiratory distress syndrome and multiorgan failure. All three patients died.

The gene sequences also indicate that these viruses may be better adapted than other avian influenza viruses to infecting mammals.

For example, the presence of Q226L in the HA protein has been associated with reduced binding to avian-like receptors bearing sialic acids linked to galactose by α-2,3 linkages found in the human lower respiratory tract,1 and potentially an enhanced ability to bind to mammalian-like receptors bearing sialic acids linked to galactose by α-2,6 linkages located in the human upper airway.

Equally troubling is that Q226L in HA has been shown to be associated with transmission of HPAI H5N1 viruses by respiratory droplets in ferrets, one of the animal models for assessing pathogenicity and transmissibility of influenza viruses.

These H7N9 viruses also possess the E627K substitution in the PB2 protein, which has also been associated with mammalian adaptation and respiratory-droplet transmission of HPAI H5N1 virus in ferrets.

This H7N9 virus is a novel reassortant with HA and neuraminidase (NA) genes from an ancestral avian H7N9 virus and the six other genes from an avian H9N2 virus.

The animal reservoir now appears to be birds, but many experts are asking whether these viruses might also be able to infect pigs, another common reservoir for zoonotic infections.

H7N9 seems to be susceptible to neuraminidase inhibitors

The viral sequence data indicate antiviral resistance to the adamantanes and susceptibility to neuraminidase inhibitors, except for an R292K mutation in the NA protein of the A/Shanghai/1/2013 virus. Because this mutation has been associated with in vitro resistance to neuraminidase inhibitors in another N9 NA subtype virus, additional analyses must be undertaken to understand its significance. It is not known whether this mutation arose *de novo* in the host or is associated with oseltamivir treatment. Ongoing surveillance is crucial to assessing the emergence and prevalence of H7N9 viruses resistant to available antivirals.

The newest influenza vaccines studies

Researchers at nine sites nationwide have begun testing in humans an investigational H7N9 avian influenza vaccine. The two concurrent Phase II clinical trials, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, are designed to gather critical information about the safety of the candidate vaccine and the immune system responses it induces when administered at different dosages and with or without adjuvants, substances designed to boost the body's immune response to vaccination.

Human cases of H7N9 influenza first emerged in China in February 2013, with the majority of reported infections occurring in the spring. As of Aug. 12, 135 confirmed human cases, including 44 deaths, have been reported by the World Health Organization. Most of these cases involved people who came into contact with infected poultry. Although no H7N9 influenza cases have been reported outside of China and the virus has not demonstrated sustained person-to-person transmission, there is concern that it could mutate to pose a much greater public health threat.

"H7N9 avian influenza virus" — like all novel influenza virus strains to which people have not been exposed — has the potential to cause widespread sickness and mortality," said NIAID Director Anthony S. Fauci, M.D. "We are now testing a vaccine candidate with and without adjuvant in an effort to prepare for and, hopefully, protect against this possibility."

The two clinical trials, which will enroll healthy adults ages 19 to 64, will evaluate an investigational H7N9 vaccine developed by Sanofi Pasteur. The candidate vaccine was made from inactivated H7N9 virus isolated in Shanghai, China in 2013. Adjuvants are being tested with the investigational vaccine because previous vaccine research involving other H7 influenza viruses has suggested that two doses of vaccine without adjuvant may not produce an immune response adequate to provide effective protection. In pandemic situations, adjuvants also can be used as part of a dose-sparing strategy, which would allow production of more doses of vaccine from the available supply of the viral antigen, thereby allowing a greater number of people to be vaccinated more quickly.

The first clinical trial, led by Mark J. Mulligan, M.D., of Emory University in Atlanta, will enroll as many as 700 study participants who will be randomly assigned to one of seven groups (up to 100 participants in each group). Each group will receive two equivalent dosages (3.75 micrograms [mcg], 7.5 mcg, 15 mcg or 45 mcg) of the candidate vaccine, approximately 21 days apart. Five of the groups will receive the vaccinations along with MF59 adjuvant, developed by Novartis Vaccines and Diagnostics.

Of these five groups, three will receive adjuvant with both vaccinations; one group of participants will receive adjuvant only with the first vaccination; and another group of participants will receive adjuvant only with the second vaccination. Two groups of participants will not receive adjuvant. The MF59 adjuvant that is being tested is also contained in the Fluad seasonal influenza vaccine currently licensed in

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Europe and Canada for use in people age 65 years or older.

The second trial, led by Lisa A. Jackson, M.D., M.P.H., of Group Health Research Institute in Seattle, will enroll as many as 1,000 participants. Each participant will be randomly assigned to one of 10 groups (up to 100 participants per group) and will receive two equivalent doses (same dosages as the other trial) of the investigational H7N9 vaccine given 21 days apart.

Seven of these groups will receive the vaccinations either with or without AS03 adjuvant, developed by GlaxoSmithKline Biologics. Two groups will receive their first vaccination with AS03 or MF59 adjuvant and then receive the alternate adjuvant at time of second vaccination. One group will receive the MF59 adjuvant at both vaccinations. The AS03 adjuvant that is being tested was used in a 2009 H1N1 influenza vaccine, Pandemrix, used in several European countries during the 2009-2010 H1N1 influenza pandemic.

In both studies, which are expected to conclude in December 2014, a panel of independent experts will closely monitor safety data at regular intervals throughout the trial.

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