ENVELOPED RNA VIRUSES
ORTHOMYXOVIRUSES

Virology Lec. For M.Sc. 2017-2018

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Orthomyxoviruses (Influenza Viruses): Introduction

- The **Orthomyxoviridae** (influenza viruses) are a major determinant of morbidity and mortality caused by respiratory disease, and outbreaks of infection sometimes occur in worldwide epidemics.

- Influenza has been responsible for millions of deaths worldwide.

- Mutability and high frequency of genetic reassortment and resultant antigenic changes in the viral surface glycoproteins make influenza viruses formidable challenges for control efforts.

Table 1 Important Properties of Orthomyxoviruses

Virion: Spherical, pleomorphic, 80–120 nm in diameter (helical nucleocapsid, 9 nm)

Composition: RNA (1%), protein (73%), lipid (20%), carbohydrate (6%)

Genome: Single-stranded RNA, segmented (eight molecules), negative-sense, 13.6 kb overall size

Proteins: Nine structural proteins, one nonstructural

Envelope: Contains viral hemagglutinin (HA) and neuraminidase (NA) proteins

Replication: Nuclear transcription; capped 5' termini of cellular RNA scavenged as primers; particles mature by budding from plasma membrane

Outstanding characteristics:

Genetic reassortment common among members of the same genus

Influenza viruses cause worldwide epidemics

- Influenza viruses are members of the **orthomyxovirus** group, which are enveloped, pleomorphic,single-stranded RNA viruses. They are classified into three major serotypes, A, B, and C, based on different ribonucleoprotein antigens.

- Influenza A viruses are the most extensively studied of the three, and much of the following discussion is based on knowledge of this type. They generally cause more severe disease and more extensive epidemics than the other types; naturally infect a wide variety of species, including mammals and birds; and have a great tendency to undergo significant antigenic changes.

- Influenza B viruses are more antigenically stable; are only known to naturally infect humans; and usually occur in more localized outbreaks.

- Influenza C viruses appear to be relatively minor causes of disease, affecting humans and pigs

Differences Among Influenza Viruses

Feature	Influenza A	Influenza B	Influenza C
Gene segments	8	8	7
Unique proteins	M2	NB	HEF
Host range	Humans, swine, avians, equines, marine mammals	Humans only	Humans, swine
Disease severity	Often severe	Occasionally severe	Usually mild
Epidemic potential	Extensive; epidemics and pandemics (antigenic drift and shift)	Outbreaks; occasional epidemics (antigenic drift only)	Limited outbreaks (antigenic drift only)

Viral Structure

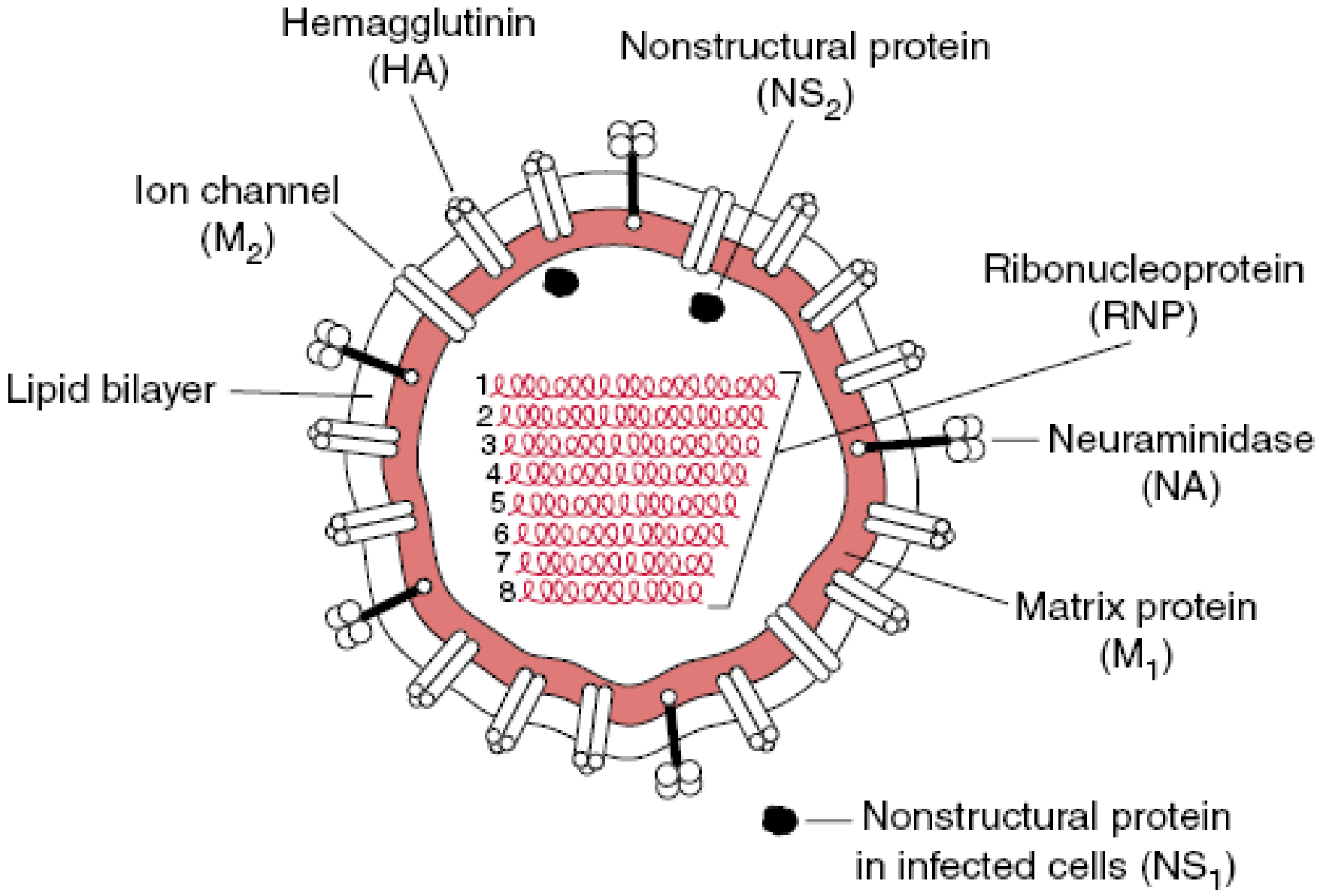
- Influenza A and B viruses each consist of a nucleocapsid containing eight segments of negative-sense, **single-stranded RNA,** which is enveloped in a glycolipid membrane derived from the host cell plasma membrane. The inner side of the envelope contains a layer of virus-specified protein (M1).

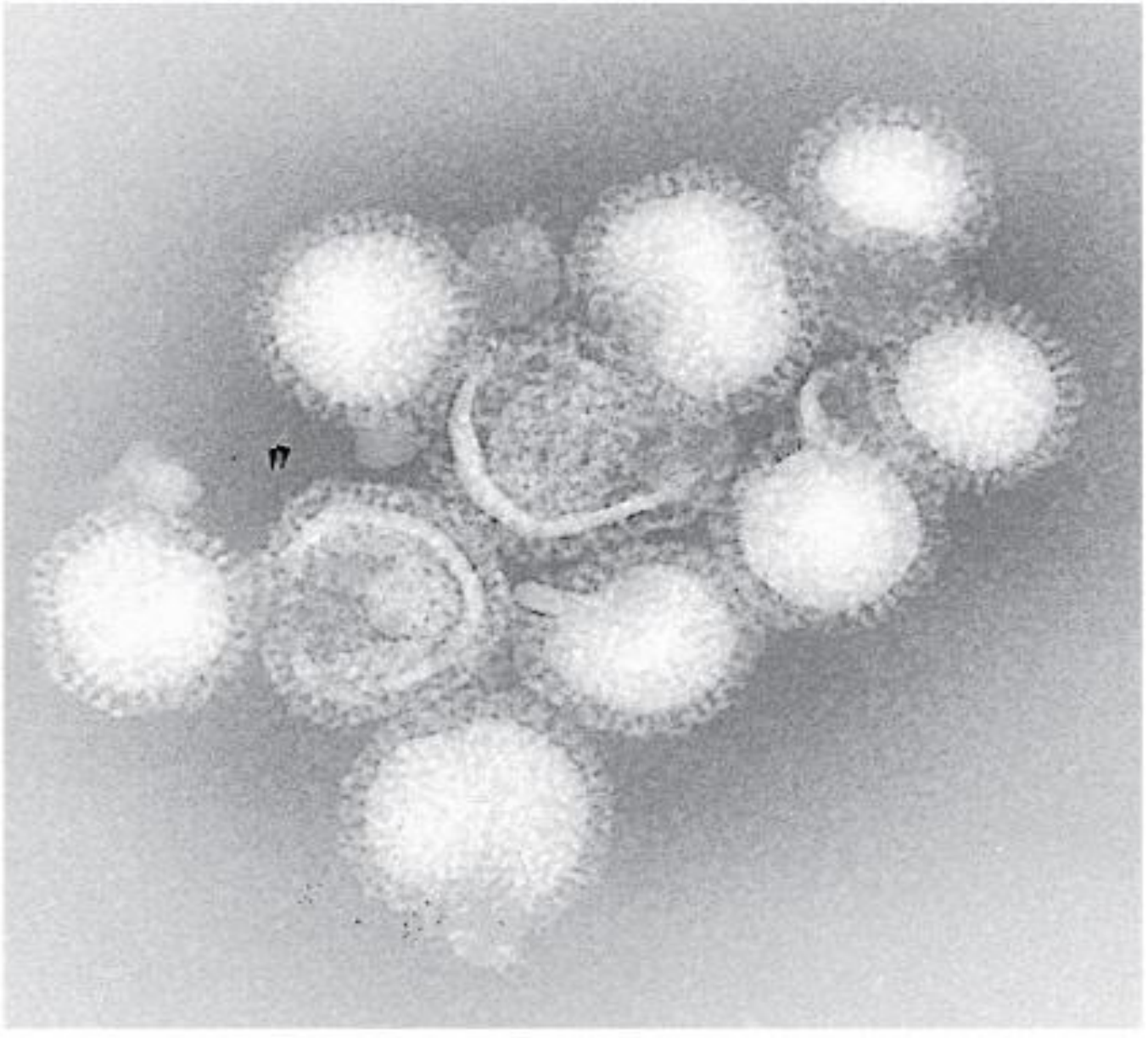
- Two virus-specified glycoproteins, hemagglutinin and neuraminidase, are embedded in the outer surface of the envelope and appear as “spikes” over the surface of the virion.

- Influenza B is somewhat similar but has a unique NB protein instead of M2.

- Influenza C differs from the others in that it possesses only seven RNA segments and has no neuraminidase, although it does possess other receptor-destroying capability.

- In addition, the hemagglutinin of influenza C binds to a cell receptor different from that for types A and B.


Hemagglutinin
(HA)
Nonstructural protein
(NS_2)
Ion channel
(M_2)
Ribonucleoprotein
(RNP)
Lipid bilayer
1
2
3
4
5
6
7
8
Neuraminidase
(NA)
Matrix protein
(M_1)
Nonstructural protein
in infected cells (NS_1)



A

Source: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA: *Jawetz, Melnick, & Adelberg's Medical Microbiology, 25th Edition*: http://www.accessmedicine.com

HA

- The virus-specified glycoproteins are antigenic and have special functional importance to the virus.

- **Hemagglutinin** is so named because of its ability to agglutinate red blood cells from certain species (eg, chickens, guinea pigs) in vitro.

- Its major biological function is to serve as a point of attachment to *N*-acetylneuraminic (sialic) acid–only containing glycoprotein or glycolipid receptor sites on human respiratory cell surfaces, which is a critical first step in initiating infection of the cell.

NA

- **Neuraminidase** is an antigenic hydrolytic enzyme that acts on the hemagglutinin receptors by splitting off their terminal neuraminic (sialic) acid. The result is destruction of receptor activity.

- Neuraminidase serves several functions. It may inactivate a free mucoprotein receptor substance in respiratory secretions that could otherwise bind to viral hemagglutinin and prevent access of the virus to the cell surface. It is important in fusion of the viral envelope with the host cell membrane as a prerequisite to viral entry.

- It also aids in the release of newly formed virus particles from infected cells, thus making them available to infect other cells.

- Type-specific antibodies to neuraminidase appear to inhibit the spread of virus in the infected host and to limit the amount of virus released from host cells.

- **Nucleocapsid** assembly takes place in the cell nucleus, but final virus assembly takes place at the plasma membrane.

- The ribonucleoproteins are enveloped by the plasma membrane, which by then contains hemagglutinin and neuraminidase. Virus “buds” are formed, and intact virions are released from the cell surface.

- The viruses replicate in the amniotic sac of embryonated hen’s eggs, where their presence can be detected by the hemagglutination test. Most strains can also be readily isolated in cell culture systems, such as primary monkey kidney cells. Some cause cytopathic effects in culture.

Classification & Nomenclature

- The standard nomenclature system for influenza virus isolates includes the following information:
- type, host of origin, geographic origin, strain number, and year of isolation.

- Antigenic descriptions of the HA and the NA are given in parentheses for type A. The host of origin is not indicated for human isolates, eg, A/Hong Kong/03/68(H3N2), but it is indicated for others, eg, A/swine/Iowa/15/30(H1N1).

- So far, 15 subtypes of HA (H1–H15) and nine subtypes of NA (N1–N9), in many different combinations, have been recovered from birds, animals, or humans. Four HA (H1–H3, H5) and two NA (N1, N2) subtypes have been recovered from humans

influenza A

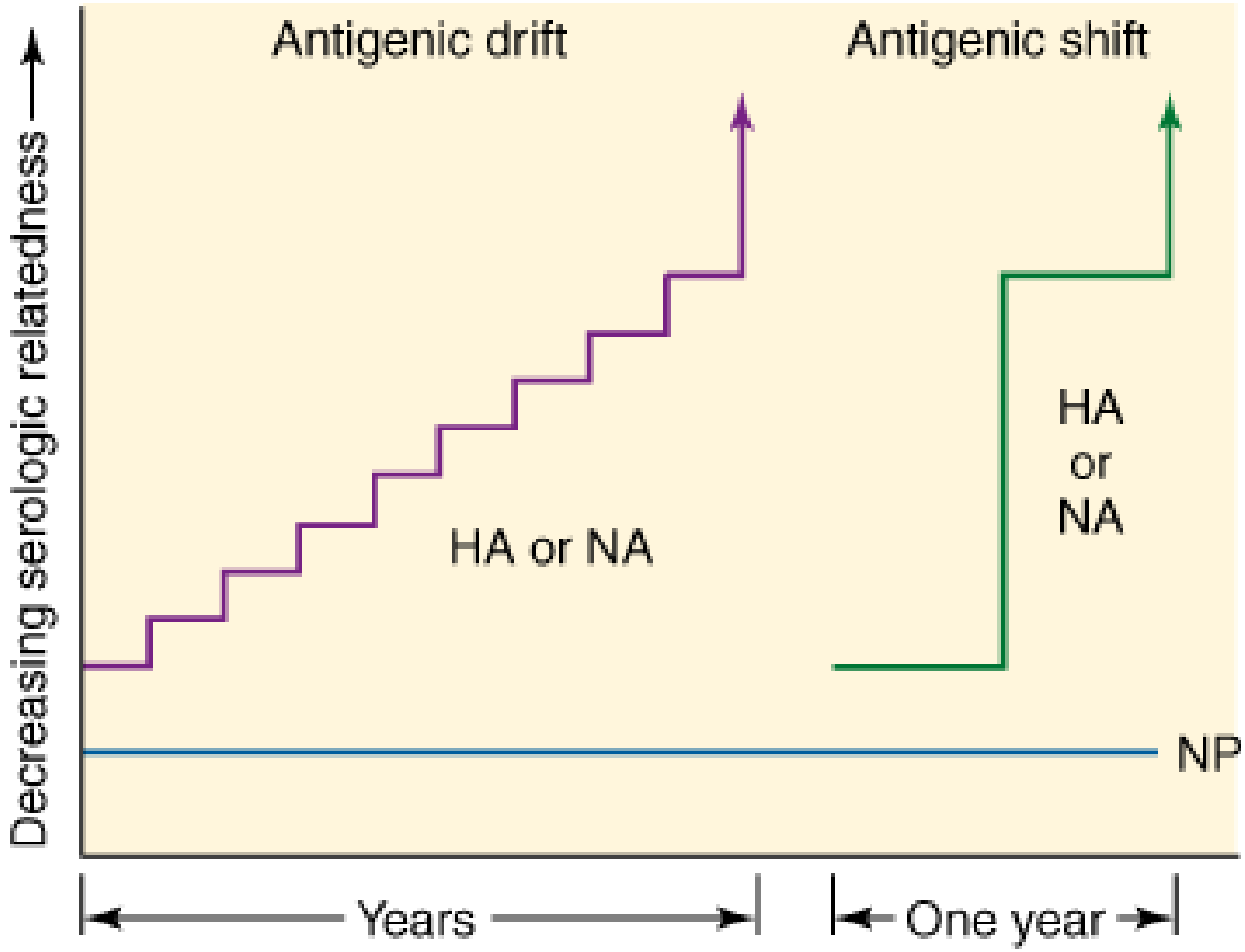
- A unique aspect of influenza A viruses is their ability to develop a wide variety of subtypes through the processes of mutation and whole-gene “swapping” between strains, called **reassortment.**

- Recombination, which occurs when new genes are assembled from sections of other genes, is thought to occur rarely, if at all. These processes result in antigenic changes called **drifts** and **shifts,** which are discussed shortly

Antigenic Drift & Antigenic Shift

- Influenza viruses are remarkable because of the frequent antigenic changes that occur in HA and NA. Antigenic variants of influenza virus have a selective advantage over the parental virus in the presence of antibody directed against the original strain. This phenomenon is responsible for the unique epidemiologic features of influenza. Other respiratory tract agents do not display significant antigenic variation.

- The two surface antigens of influenza undergo antigenic variation independent of each other. Minor antigenic changes are termed **antigenic drift;** major antigenic changes in HA or NA, called **antigenic shift,** result in the appearance of a new subtype. Antigenic shift is most likely to result in an epidemic.



Source: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA: *Jawetz, Melnick, & Adelberg's Medical Microbiology, 25th Edition*: http://www.accessmedicine.com

Antigenic Drift

- Antigenic drift is due to the accumulation of point mutations in the gene, resulting in amino acid changes in the protein.

- Sequence changes can alter antigenic sites on the molecule such that a virion can escape recognition by the host's immune system. The immune system does not cause the antigenic variation, but rather functions as a selection force that allows new antigenic variants to expand.

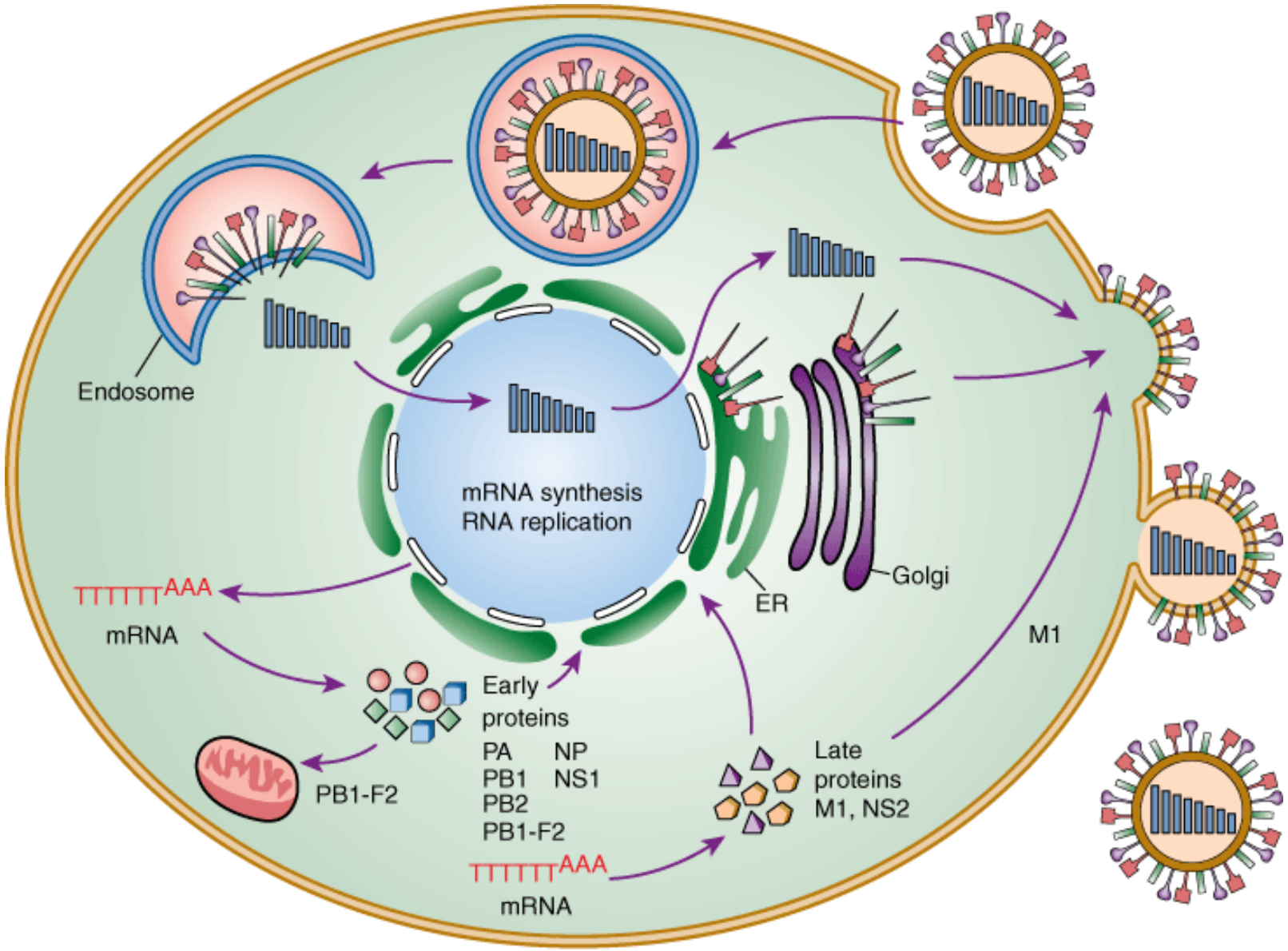
- A variant must sustain two or more mutations before a new, epidemiologically significant strain emerges.

Antigenic Shift

- In contrast to the frequently occurring mutations that cause antigenic drift among influenza A strains, major changes (>50%) in the nucleotide sequences of the H or N genes can occur suddenly and unpredictably. These are referred to as antigenic shifts
- Antigenic shift reflects drastic changes in the sequence of a viral surface protein, changes too extreme to be explained by mutation.
- The segmented genomes of influenza viruses reassort readily in doubly infected cells.
- The mechanism for shift is genetic reassortment between human and avian influenza viruses.
- Influenza B and C viruses do not exhibit antigenic shift because few related viruses exist in animals.

Influenza Virus Replication

- Influenza is unusual among non-oncogenic RNA viruses because all of its RNA transcription and replication occur in the nucleus of infected cells.
- The viral multiplication cycle proceeds rapidly.
- There is the shut-off of host cell protein synthesis by about 3 hours postinfection, permitting selective translation of viral mRNAs.
- New progeny viruses are produced within 8–10 hours.



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Viral Attachment, Penetration, & Uncoating

- The virus attaches to cell-surface sialic acid via the receptor site located on the top of the large globule of the HA. Virus particles are then internalized within endosomes by a process called receptor-mediated endocytosis.

- The next step involves fusion between the viral envelope and cell membrane, triggering uncoating. The low pH within the endosome is required for virus-mediated membrane fusion that releases viral RNPs into the cytosol. Acid pH causes a conformational change in the HA structure to bring the HA2 "fusion peptide" in correct contact with the membrane.

- The M_2 ion channel protein present in the virion permits the entry of ions from the endosome into the virus particle, triggering the conformational change in HA. Viral nucleocapsids are then released into the cell cytoplasm.

Transcription & Translation

- The mRNAs are produced from viral nucleocapsids. The virus-encoded polymerase, consisting of a complex of the three P proteins, is primarily responsible for transcription.

- Its action must be primed by scavenged capped and methylated 5' terminals from cellular transcripts that are newly synthesized by cellular RNA polymerase II.

- This explains why influenza virus replication is inhibited by dactinomycin and -amanitin, which block cellular transcription, whereas other RNA viruses are not affected because they do not use cellular transcripts in viral RNA synthesis.

Budding

- Progeny virions bud off the cell. During this sequence of events, the HA is cleaved into HA1 and HA2 if the host cell possesses the appropriate proteolytic enzyme.

- The NA removes terminal sialic acids from cellular and viral surface glycoproteins, facilitating release of virus particles from the cell and preventing their aggregation.

- Many of the particles are not infectious. Particles sometimes fail to encapsidate the complete complement of genome segments; frequently, one of the large RNA segments is missing. These noninfectious particles are capable of causing hemagglutination and can interfere with the replication of intact virus.

Clinical Feature

- Influenza virus types A and B typically cause more severe symptoms than influenza virus type C.

- The typical illness is characterized by an abrupt onset (over several hours) of fever, diffuse muscle aches and chills. This is followed within 12 to 36 hours by respiratory signs, such as rhinitis, cough, and respiratory distress.

- The acute phase usually lasts 3 to 5 days, but a complete return to normal activities may take 2 to 6 weeks. Serious complications, especially pneumonia, are common

Complications of Influenza

- Occasionally, patients develop a progressive infection that involves the tracheobronchial tree and lungs. In these situations, pneumonia, which can be lethal, is the result.

- Other unusual acute manifestations of influenza include central nervous system (CNS) dysfunction, myositis, and myocarditis.

- In infants and children, a serious complication known as Reye's syndrome may develop 2 to 12 days after onset of the infection. It is characterized by severe fatty infiltration of the liver and cerebral edema. The risk is enhanced by exposure to salicylates, such as aspirin.

- The most common and important complication of influenza virus infection is bacterial superinfection. Such infections usually involve the lung, but bacteremia with secondary seeding of distant sites can also occur. The bacteria most commonly involved include *Streptococcus pneumoniae, Haemophilus influenzae,* and *Staphylococcus aureus.*

EPIDEMIOLOGY

- Humans are the major hosts of the influenza viruses, and severe respiratory disease is the primary manifestation of infection.

- **Direct droplet spread** is the most common mode of transmission. Influenza infections in temperate climates tend to occur most frequently during midwinter months. Major epidemics of influenza A usually occur at 2- to 3-year intervals, and influenza.

- The typical epidemic develops over a period of 3 to 6 weeks and can involve 10% of the population. Illness rates may exceed 30% among school-aged children, residents of closed institutions.

PATHOGENESIS

- Influenza viruses have a predilection for the respiratory tract, and viremia is rarely detected. They multiply in ciliated respiratory epithelial cells, leading to functional and structural ciliary abnormalities.

- This is accompanied by a switch-off of protein and nucleic acid synthesis in the affected cells, the release of lysosomal hydrolytic enzymes, and desquamation of both ciliated and mucus-producing epithelial cells. Thus, there is substantial interference with the mechanical clearance mechanism of the respiratory tract.

- The process of programmed cell death (apoptosis) results in the cleavage of complement components, leading to localized inflammation. Early in infection, the primary chemotactic stimulus is directed toward mononuclear leukocytes, which constitute the major cellular inflammatory component. The respiratory epithelium may not be restored to normal for 2 to 10 weeks after the initial insult

Net result of infection

- The net result of these effects is that, on entry into the respiratory tract, the viruses cause cell damage, especially in the respiratory epithelium, which elicits an acute inflammatory response and impairs mechanical and cellular host responses.

- This damage renders the host highly susceptible to invasive bacterial **superinfection.**

- Recovery from infection begins with interferon production, which limits further virus replication, and with rapid generation of natural killer cells. Shortly thereafter, class I major histocompatibility complex (MHC)–restricted cytotoxic T cells appear in large numbers to participate in the lysis of virus-infected cells and, thus, in initial control of the infection.

- This is followed by the appearance of local and humoral antibody along with an evolving, more durable cellular immunity. Finally, there is repair of tissue damage

IMMUNITY

- Typically, patients respond to infection within a few days by producing antibodies directed toward the group ribonucleoprotein antigen, the hemagglutinin, and the neuraminidase.

- Peak antibody titer levels are usually reached within 2 weeks of onset and then gradually wane over the following months to varying low levels.

- Antibody to the ribonucleoprotein appears to confer little or no protection against reinfection. Antihemagglutinin antibody is considered the most protective; it has the ability to neutralize virus on reexposure. Antibody to neuraminidase antigen is not as protective as antihemagglutinin antibody but plays a role in limiting virus spread within the host

- However, such immunity is relative, and quantitative differences in responsiveness exist between individuals. Furthermore, antigenic shifts and drifts often allow the virus to subvert the antibody response on subsequent exposures.

Laboratory Diagnosis

- Clinical characteristics of viral respiratory infections can be produced by many different viruses.

- Consequently, diagnosis of influenza relies on identification of viral antigens or viral nucleic acid in specimens, isolation of the virus, or demonstration of a specific immunologic response by the patient.

- Nasal washings, gargles, and throat swabs are the best specimens for diagnostic testing and should be obtained within 3 days after the onset of symptoms

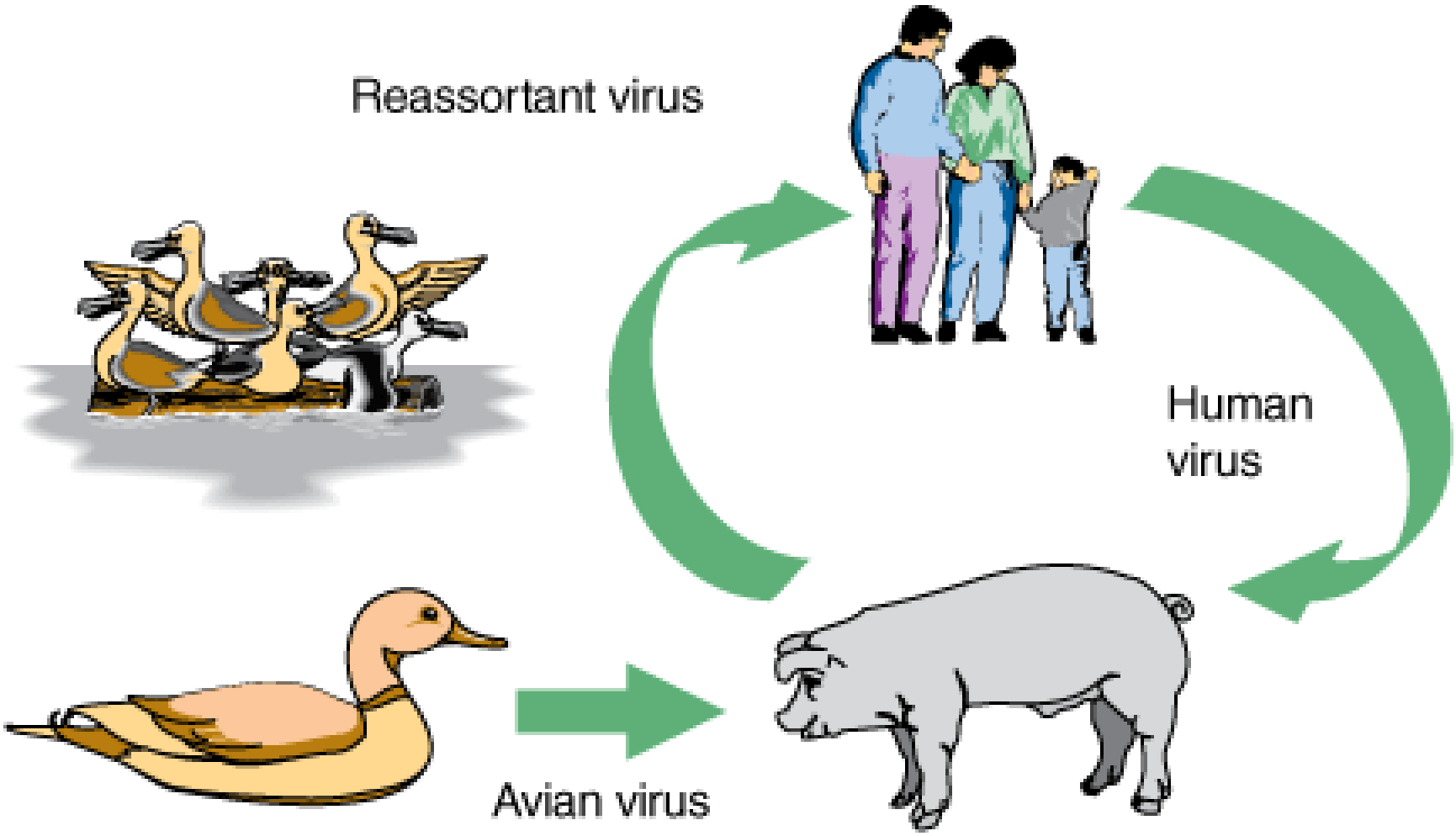
Avian Influenza

- Sequence analyses of influenza A viruses isolated from many hosts in different regions of the world support the theory that all mammalian influenza viruses derive from the avian influenza reservoir.

- Of the 15 HA subtypes found in birds, only a few have been transferred to mammals (H1, H2, H3, and H5 in humans; H1 and H3 in swine; and H3 and H7 in horses). The same pattern holds for NA; nine NA subtypes are known for birds, only two of which are found in humans (N1, N2).

- The influenza viruses do not appear to undergo antigenic change in the birds, perhaps because of their brief life span. This means the genes that caused previous influenza pandemics in humans still exist unchanged in the aquatic bird reservoir.

- It is likely that avian influenza is a waterborne infection, moving from wild to domestic birds and pigs.
- To date, all human pandemic strains have been reassortants between avian and human influenza viruses.
- Evidence supports the model that pigs serve as mixing vessels for reassortants as their cells contain receptors recognized by both human and avian viruses.
- The pandemic strain of 2009 was a novel reassortant that contained swine-origin viral genes as well as those from avian and human influenza viruses. School-age children are the predominant vectors of influenza transmission. Crowding in schools favors the aerosol transmission of virus, and children take the virus home to the family.



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Avian Influenza is a time bomb!

- Of about 425 laboratory-confirmed human cases, by May 2009 more than half have been fatal.
- So far, isolates from human cases have contained all RNA gene segments from avian viruses, indicating that, in those infections, the avian virus had jumped directly from bird to human.
- All evidence to date indicates that the only way of H5N1 infection transmission to humans is by close contact with diseased birds (no human to human transmission).
- The concern is that, given enough opportunities, the highly pathogenic H5N1 avian influenza virus will acquire the ability to spread efficiently among humans and be sustained in humans, either by further reassortment or by adaptive mutation. This would result in a devastating influenza pandemic

Prevention & Treatment by Drugs

- Amantadine hydrochloride and an analog, rimantadine, are M_2 ion channel inhibitors for systemic use in the treatment and prophylaxis of influenza A.

- The NA inhibitors zanamivir and oseltamivir were approved in 1999 for treatment of both influenza A and influenza B.

- To be maximally effective, the drugs must be administered very early in the disease. Resistant viruses emerge more frequently during therapy with M_2 inhibitors than with NA inhibitors and more frequently in children than adults..

Prevention & Control by Vaccines

- Inactivated viral vaccines are the primary means of prevention of influenza. However, certain characteristics of influenza viruses make prevention and control of the disease by immunization especially difficult.

- Existing vaccines are continually being rendered obsolete as the viruses undergo antigenic drift and shift. Surveillance programs constantly monitor subtypes of influenza circulating around the world to promptly detect the appearance and spread of new strains.

- Several other problems are worthy of mention. Although protection can reach 70–100% in healthy adults, frequency of protection is lower (30–60%) among the elderly and among young children.

- Inactivated viral vaccines usually do not generate good local IgA or cell-mediated immune responses. The immune response is influenced by whether the person is "primed" by having had prior antigenic experience with an influenza A virus of the same subtype

- Inactivated influenza A and B virus vaccines are licensed for parenteral use in humans.
- World Health Organization make recommendations each year about which strains should be included in the vaccine.
- The vaccine is usually a cocktail containing one or two type A viruses and a type B virus of the strains isolated in the previous winter's outbreaks.

Live-Virus Vaccines

- A live-virus vaccine must be attenuated so as not to induce the disease it is designed to prevent. In view of the constantly changing face of influenza viruses in nature and the extensive laboratory efforts required to attenuate a virulent virus, the only feasible strategy is to devise a way to transfer defined attenuating genes from an attenuated master donor virus to each new epidemic or pandemic isolate.

- A cold-adapted donor virus, able to grow at 25°C but not at 37°C—the temperature of the lower respiratory tract—should replicate in the nasopharynx, which has a cooler temperature (33°C). A live attenuated, cold-adapted, temperature-sensitive, trivalent influenza virus vaccine administered by nasal spray was licensed in the United States in 2003. It was the first live-virus influenza vaccine approved in the United States. This vaccine induces both humeral and CMI.

Use of Influenza Vaccines

- The only contraindication to vaccination is a history of allergy to egg protein. Since vaccine strains are grown in eggs, some egg protein antigens are present in the vaccine.

- Annual influenza vaccination is recommended for all children aged 6 months to 18 years and for high-risk groups. These include individuals at increased risk of complications associated with influenza infection (those with either chronic heart or lung disease, including children with asthma, or metabolic or renal disorders; residents of nursing homes; persons infected with the human immunodeficiency virus [HIV]; and those 65 years of age and older) and persons who might transmit influenza to high-risk groups (medical personnel, employees in chronic care facilities, household members). The live-virus intranasal vaccine is not currently recommended for individuals in the high-risk groups