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Influenza Virus Infections

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Glossary

+ Strand RNA Same polarity as the messenger RNA.

− Strand RNA Opposite polarity of the messenger RNA.

Antigenic drift Changes in antigenic epitopes due to mutations.

Antigenic shift Major change in antigenic properties usually due to RNA reassortment and mutations.

Budding Assembly, maturation, and release of virus particles from cell membrane.

Core Internal component including ribonucleoprotein (RNP) and polymerase complex.

Epidemics Local outbreaks usually restricted to a limited geographical region.

Glycosylation Attachment of carbohydrates to protein backbone.

Hemadsorption Adsorption of erythrocytes to virus-infected cells.

Hemagglutination (HA) Agglutination of erythrocytes due to lattice formation, an assay used for quantifying virus particles.

Hemagglutination inhibition (HAI) Assay used for measuring antibody titer against flu virus.

Influenza Acute respiratory disease caused by viruses belonging to the Orthomyxovirus group.

Pandemics Widespread global outbreaks of a disease; influenza pandemics are caused by antigenic shift.

Reassortment Exchange of viral genes (RNA) usually occur when a single cell is infected by two or more viruses and may lead to antigenic shift.

Replication Synthesis of full-length + and − strand viral RNA.

Transcription Synthesis of messenger RNA (mRNA).

Tropism Affinity of a virus for a specific organ, tissue, or cell.

Virulence Ability of a virus to cause disease. Relative virulence is determined by the severity of disease and spread among the population.

Introduction

Influenza or flu is a contagious, febrile viral disease. It is often accompanied by inflammation of the upper and lower respiratory tract, myalgia, gastrointestinal disorders, and neurological disorders such as headache, prostration, and insomnia. Influenza may lead to bronchitis, pneumonia, hospitalization, and death, particularly in high-risk and elderly people living in nursing homes. Influenza is caused by viruses belonging to orthomyxovirus group (Family Orthomyxoviridae; 'ortho' = true; 'myxo' = affinity for mucoproteins) (Nayak, 1997).

Virus

Influenza viruses (IVs) are enveloped, segmented, negative-stranded (−) RNA viruses containing helical nucleocapsids (also called ribonucleoprotein or RNP). An IV particle is usually spherical and of approximately 110 nm in diameter (Figures 1, 2(a), 2(b), 3(a), and 3(b)), although some particles can be filamentous and segmented (Figures 2(c), 3(c), and 3(d)). The virus envelope is a mosaic lipid bilayer containing spikes on the outside and matrix protein (M1) on the inside. The spikes, both hemagglutinin (HA) and neuraminidase (NA), are anchored in the lipid bilayer. The genetic material (genome) of IV consists of single, negative-stranded (−) RNA segments complexed with nucleoprotein (NP) forming RNP. The virus genome contains eight distinct RNA segments (seven RNA segments in influenza C virus), each of which encodes at least one (sometimes two or more) protein. The viral RNAs are of negative polarity (−, minus strand), i.e., opposite polarity of mRNAs and cannot function as the template for protein synthesis. Viral mRNAs (+, positive polarity) are made after infection to serve as a template for protein synthesis. Therefore, each infectious IV particle must carry RNA-dependent RNA polymerase (RDRP or transcriptase, replicase) and the naked IV RNA is not infectious. RDRP is a heterotrimer of PB1, PB2, and PA proteins (3P complex). The replication and transcription of viral RNA occur inside the nucleus of the infected cells, whereas assembly/budding leading to virion formation takes place on the plasma membrane (Figure 4). Complete virus particles are not found inside the infected cell.

Nomenclature and Classification

IVs are divided into three types, namely, influenza type A, type B, and type C, based on immunologic and biologic properties. Types A and B viruses possess eight RNA segments, whereas type C viruses possess seven RNA segments. Type specificity is based on the antigenic nature of two major internal antigens: NP and M1 (matrix protein), serological specificity of which is determined by complement fixation assay or enzyme-linked immunosorbant assay (ELISA). All type A viruses have similar M1 and NP proteins, which are different from those of type B or type C viruses. However, currently, nucleic acid assays

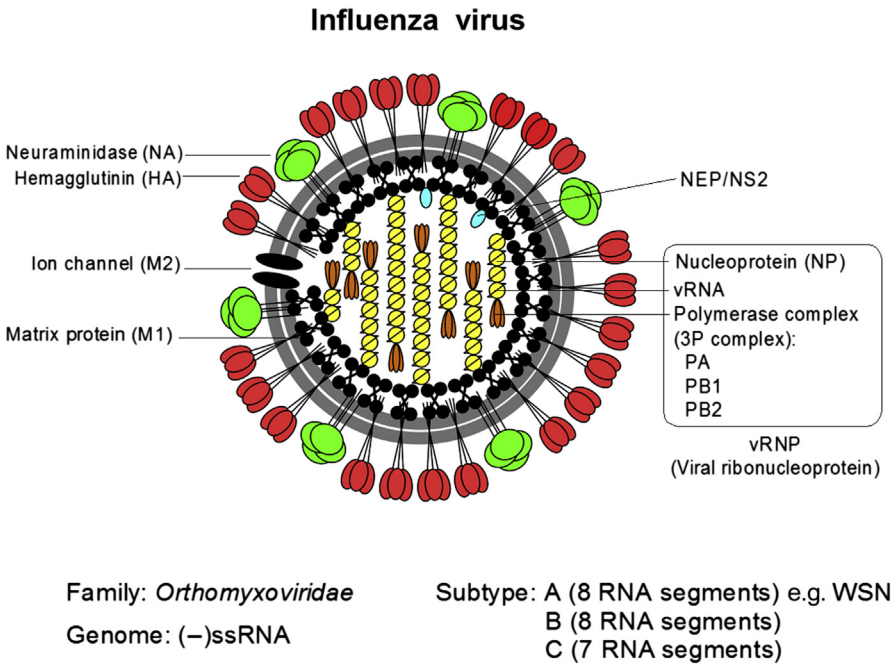

Figure 1 Structure of influenza viruses. Schematic presentation of influenza virus (IV). Membrane proteins: HA (hemagglutinin), NA (neuraminidase), MI (matrix), M2 (proton channel); core proteins: NP (nucleoprotein), PA (polymerase acidic protein), PB1, PB2 (polymerase basic proteins 1 and 2); helical RNP (ribonucleoprotein or nucleocapsid).

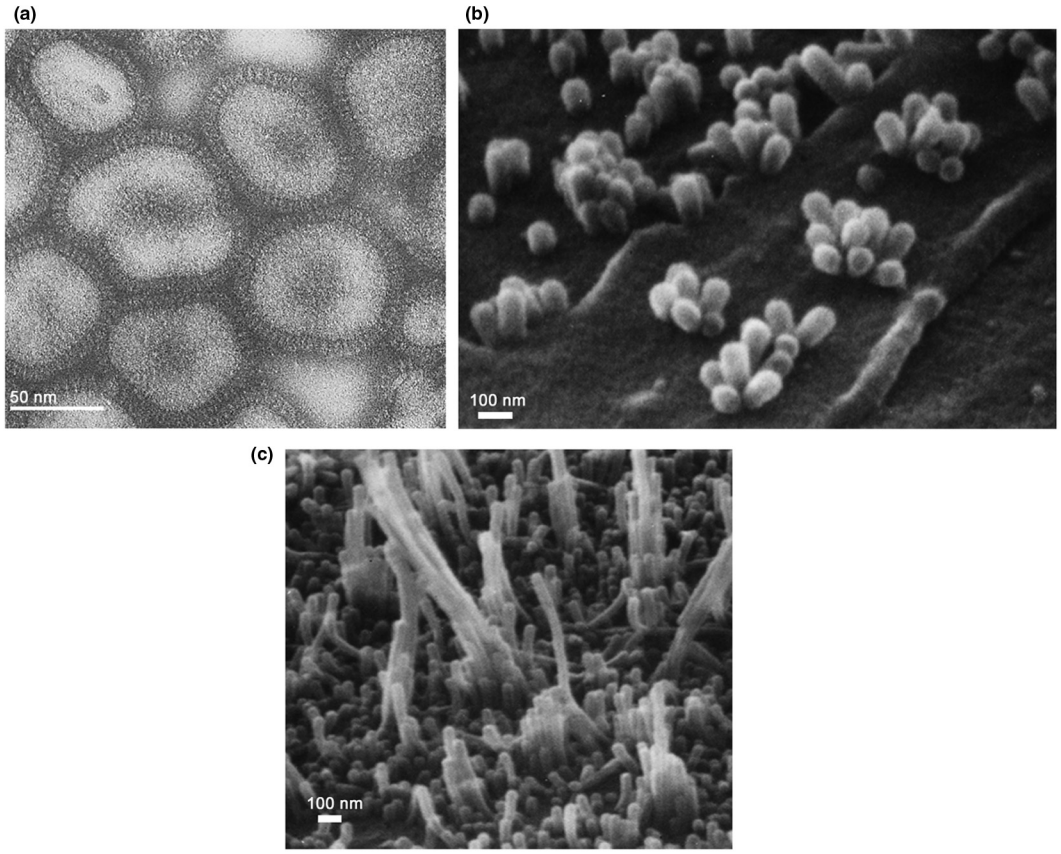

Figure 2 Morphology of influenza viruses. (a) Transmission electron micrograph (EM) of influenza virus (IV) type A. Bar 50 nm. (b) Scanning EM of spheroidal IV buds in clusters on cell surface. Bar 100 nm. (c) Scanning EM of filamentous IV buds on the cell surface. Bar 100 nm. Figure 2(a) was provided by K.G. Murti of St. Jude Children's Research Hospital of Memphis, Tennessee; Scanning EM (Figures 2(b) and 2(c)) were provided by David Hockley of the National Institute for Biological Standards and Control at Hertfordshire, UK.

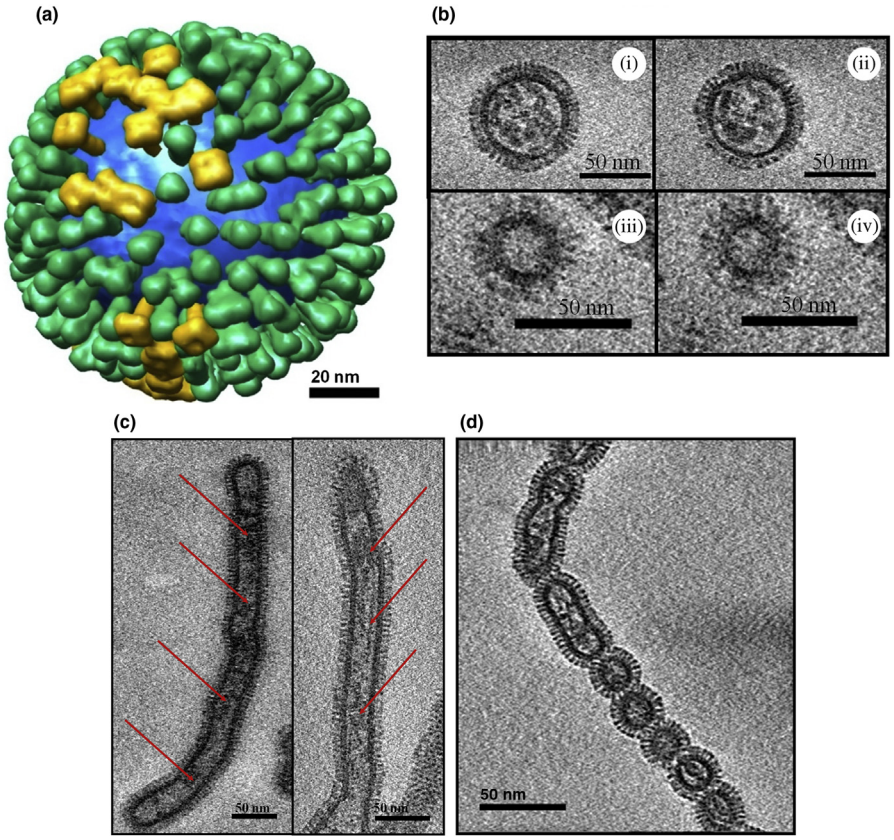

Figure 3 Structure of IV by cryoET. (a) Reconstruction of spheroidal IV from cryoET. HA (green) and NA (gold) spikes and lipid bilayer (blue) reconstructed from cryoET analysis. Bar 20 nm. (b) CryoET of Udorn (H3N2) particles with or without RNP. Spheroidal IV particles with or without RNP cores at varying heights in the Z direction. Boxes (i) and (ii) show the same particle with RNP at varying heights; RNP is found throughout the particle. In contrast, boxes (iii) and (iv), show the same particle without RNP core at varying heights. No electron-dense RNP was found throughout the entire particle. Bar 50 nm. (c) RNP distribution in H3N2 filamentous particles. Two different filamentous viruses show the electron dense RNP (red arrows) throughout the particle inside. Bar 50 nm. (d) Variant filamentous form of H3N2 released virions showing multiple spheroidal particles attached to each other forming a chain. Bar 50 nm. Figure 3(a) reproduced from Harris, A., Cardone, G., Winkler, D.C., Heymann, J.B., Brecher, M., White, J.M., Steven, A.C., 12 December 2006. Influenza virus pleiomorphy characterized by cryoelectron tomography. Proc. Natl. Acad. Sci. USA 103 (50), 19123–19127 with permission; Figures 3(b)–3(d) were reproduced from Nayak, D.P., Sakar, S., Balogun, R.A., Lee, G., Zhou, Z.H., 2013. Structure, disassembly, assembly and budding of influenza viruses. Text Book of Influenza. In: Webster, R.G., Braciale, T.J., Monto, A.S., et al. (Eds), Wiley-Blackwell, with permission.

including polymerase chain reaction (PCR) and genome sequencing are used to identify the type and subtype specificity as well as subtle differences among the virus strains including genetic mutations predicting evolutionary and pathogenic behavior.

Subtype specificity is determined serologically by the nature of the major envelope antigens, namely, HA and NA. Different subtypes occur only among the influenza type A viruses found in humans and other warm-blooded animals. According to the present nomenclature, 18 HA subtypes (H1 to H18) and 11 NA subtypes (N1 to N11) have been found among the influenza type A viruses including the newly discovered IVs in Peruvian bats (Tong et al., 2013). Individual virus isolates are identified by subtype specificity e.g., H1N1, H2N2, etc. (H1N1 denotes HA of subtype 1 and NA of subtype 1). Of all the possible combinations, only three subtypes H1N1, H2N2, and H3N2 are known to cause human epidemics and pandemics. The apparent reason of this restricted subtype specificity in humans is not clear, although receptor specificity of human respiratory epithelial cells may be a contributing factor. Influenza type B and type C viruses, found only in humans, do not exhibit any subtype variation, and cause milder disease compared to type A viruses. Usually, IVs are species specific in causing epidemics (i.e., IVs of pigs cause epidemics in pigs not in humans), although they can infect other species in a limited way. For example, farmers working with animals have been found to possess antibodies against chicken, swine, or equine viruses, indicating that they were infected with these viruses. In recent years, H5N1, and H7N9, highly pathogenic avian viruses, which do not spread among humans, can infect and cause fatal disease in humans.

Because of the epidemiological nature of the disease and possible emergence of new pandemic viruses, the World Health Organization (WHO) in collaboration with national organizations such as Centers for Disease Control (CDC), National

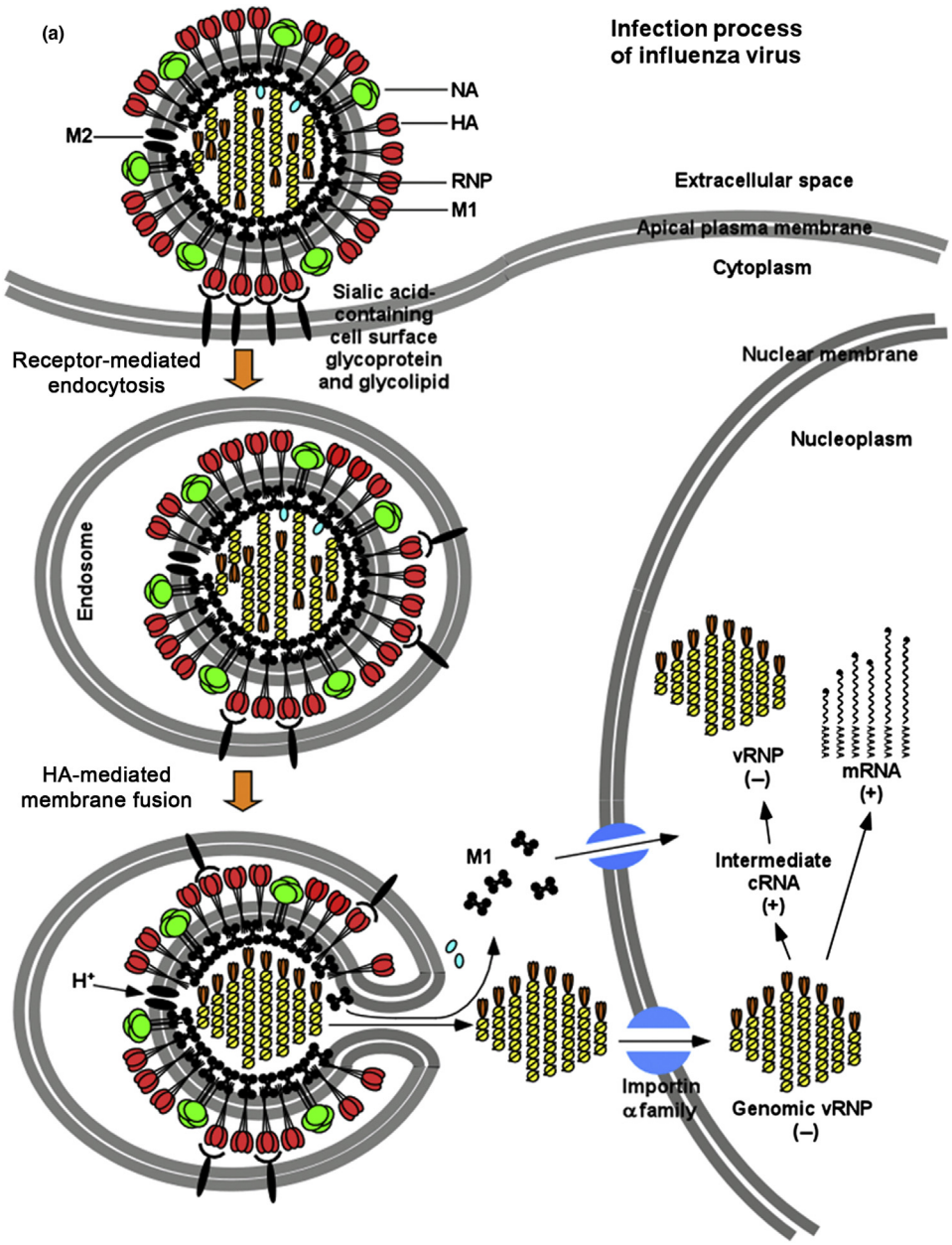

Figure 4 Schematic illustration of influenza virus infectious cycle. (a) Schematic diagram showing attachment, entry, and uncoating. Attachment is mediated through HA (red) and SA receptor; entry into the cell via endosome; HA-mediated fusion of virus and endosomal membrane at low pH; release of RNP, transport of RNP into the nucleus, transcription (mRNA synthesis) and replication (cRNA and vRNA synthesis) of RNP in the nucleus. (b) Schematic representation of nuclear exit of RNP; transport and assembly of viral components on the plasma membrane; budding of influenza virus. (c) Virus buds at the cell surface by ET. At 12 hpi WSN-infected MDCK cells were processed for thin section and examined by ET. This picture represents one slice through inner core of the virus buds. Parallel arrangement of the RNPs inside the bud perpendicular to cell surface is seen. The bud neck (⇒) shows gaps indicating possible absence of M1. HA and NA spikes are seen on the bud envelope. Reproduced from Nayak, D.P., Balogun, R.A., Yamada, H., Hong Zhou, H., Barman, S., 2009. Influenza virus morphogenesis and budding. Virus Res. 143, 147–161, with permission.

Institutes Health, and others throughout the world have organized worldwide influenza surveillance program. Consequently, a vast number of IV strains are isolated every year from humans and variety of animal species including chickens, ducks, pigs and horses. Migratory ducks and waterfowls are often promiscuous to infection by many subtypes of IV and thus serve as reservoirs for genetic reassortment and spread of IVs. A uniform system of nomenclature is followed in identifying these isolates. This includes type specificity of the virus/place of isolation/isolate number/year of isolation and is accompanied with subtype specificity when applicable. Some examples are A/Puerto Rico/8/34 (H1N1),

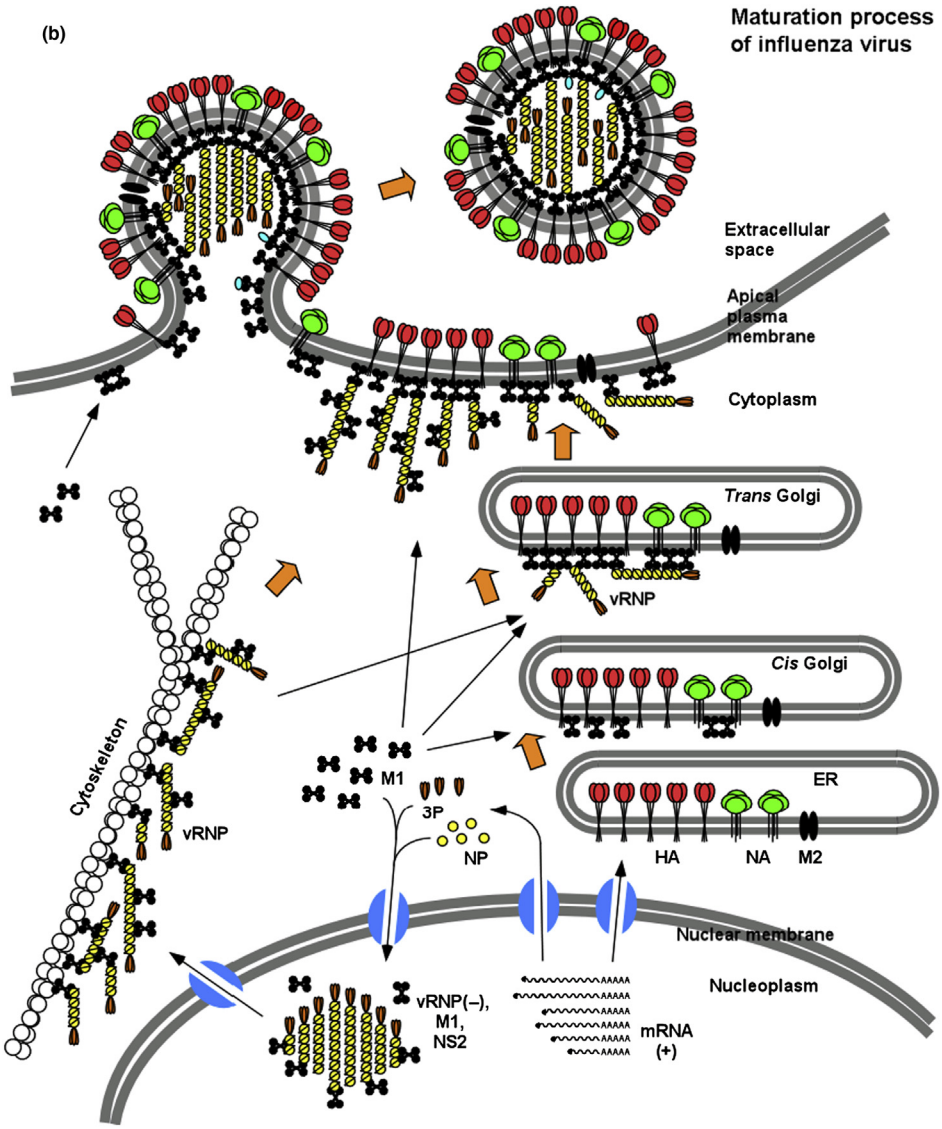

Figure 4 (*continued*).

A/California/7/2009 (H1N1), B/Great Lakes/1/54, and C/Paris/1/67. In some older prototype strains, person's name has been used instead of the place of isolation and sometimes the isolate number is missing, e.g., A/WS/34, B/Lee/40, C/Cal/78. However, the present nomenclature system fails to identify the epidemiological behavior and host of origin such as human, swine, equine, chicken, or duck.

Structure and Composition

Structure

In general, IVs are enveloped particles with surface projections or spikes, pleomorphic and roughly spheroidal (~110 nm in diameter) (Figures 1, 2(a), and 3(a)) but some are filamentous (1 μm or longer) (Figures 2(c), 3(c), and 3(d)). A schematic presentation of a typical spheroidal IV is shown (Figure 1). Usually laboratory adapted viruses, such as A/PR/8, and A/WSN/33 viruses, exhibit spheroidal/ellipsoidal morphology (Figures 2(a), 2(b), 3(a), and 3(b)), whereas fresh field isolates are more filamentous. However, some virus strains such as A/Udorn/72 (H3N2) exhibit elongated/filamentous morphology (Figures 2(c), 3(c), and 3(d)) even after many passages in the laboratory.

Virus morphology has been examined by transmission (Figure 2(a)), scanning electron microscopy (Figures 2(b) and 2(c)), and recently by cryoelectron tomography (cryoET) (Figure 3). CryoET has been used to examine both the surface and the internal content of frozen hydrated virus particle in its native state and reconstruct its three-dimensional (3D) structure (Figure 3(a)) by combining the different tilt images of the same particle. CryoET analyses show the presence of approximately 300 HA (13 nm, triangular in shape) and 40 NA (14 nm, square in shape) spikes on the surface of spheroidal particles (Harris et al., 2006) with 7 + 1 RNP core (Noda and

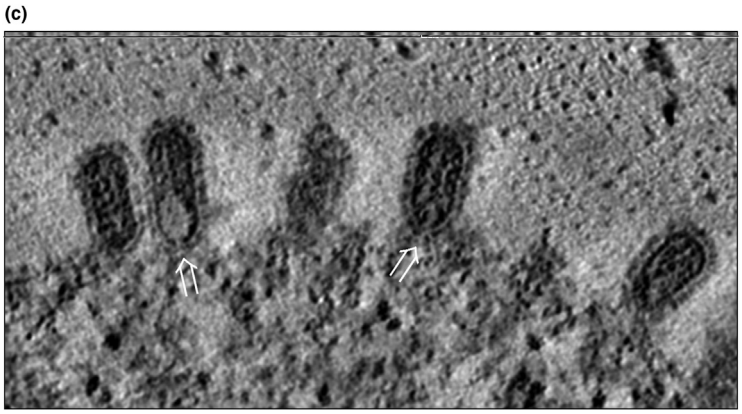

Figure 4 (*continued*).

Kawaoka, 2010). Influenza virions possess three subviral components, namely, envelope, matrix layer (M1) underneath the lipid bilayers, and RNP core (**Figure 1**).

Envelope

The envelope of influenza A virus consists of a lipid bilayer containing three virally encoded transmembrane (TM) proteins HA, NA, and M2 on the outside and M1 underneath the membrane. The lipid bilayer is mosaic containing both cholesterol-enriched lipid rafts and nonraft lipids, selectively derived from the host cell membrane. HA and NA are anchored in the lipid rafts, whereas M2, although a cholesterol-binding protein, is associated with nonraft lipids. HA is the major envelope protein (~80%) and forms the trimeric spikes with receptor-binding sites and epitopes for neutralizing antibodies. NA, the second most abundant (~17%) envelope protein, forms the mushroomlike tetrameric spikes. NA removes the cell surface sialic acid (SA) receptor and plays a critical role in releasing progeny virus particles. The third envelope protein, the M2 tetramer, is a proton selective ion channel. Although M2 is a minor component (~16–20 molecules/virion), it plays a critical role in disassembly as well as in virus release (Section Infectious Cycle).

HA polypeptide of influenza types A and B possesses around 566 and 584 amino acids (aa), respectively. In influenza C virus, a single transmembrane protein of 654 aa possesses both the receptor-binding (hemagglutination) and receptor-destroying (esterase) activity and is referred to as HE protein. HA is a type I TM glycoprotein in which the N-terminal signal sequence is removed by signal peptidase during translocation into the endoplasmic reticulum (ER) and therefore is absent in the mature HA. The C-terminal hydrophobic domain functions as a transmembrane domain (TMD) anchoring the protein into the lipid bilayer. HA is further cleaved at the internal hydrophobic domain to generate HA1 and HA2, both held together by a disulfide bond. The hydrophobic domain becomes the N-terminus of HA2. This cleavage is host cell protease dependent and occurs during the transport of HA through the membrane trafficking pathway. The cleavage of HA is not required for intracellular transport, maturation (trimmer formation or glycosylation), or assembly and budding of virus particles, or binding or entry or for hemagglutination. However, the cleavage of HA and free N-terminus of HA2 are critical for virus infectivity and fusion of the virus with the endosomal membrane at low pH (Section Infectious Cycle). Virus particles containing the uncleaved HA are not infectious, although they can bind to the cell receptor and be internalized into the endosomes. In vitro treatment of IV particles containing the uncleaved HA with a protease, such as trypsin, generates HA1 and HA2 and renders noninfectious particles infectious. The sequence specificity of the cleavage peptide at the junction of HAl and HA2 varies for different viruses but many of the structural features are conserved among the HAs found in different virus isolates. The presence of multiple basic residues like arginine and lysine render HA highly cleavable by furinlike host proteases. HA contains a number of N-linked oligosaccharides, but their number, location, and composition vary with different subtypes and even among different strains of the same subtype. Oligosaccharides provide the structural stability, protection against host proteases, and play an important role in immune-modulation of epidemic viruses. HA is present on virus particles and on the surface of the virus-infected cells. HA binds to erythrocytes causing hemagglutination and hemadsorption, assays used for quantification of virus particles and virus-infected cells, respectively.

Trimeric HA spikes are rod shaped. The 3D structure of HA spike shows two distinct structural domains: (1) a triple-stranded coiled coil fibrous stalk extending from the viral or cellular membrane and (2) globular head at the distal end of the stalk consisting of an eight-stranded β sheet and possessing the receptor binding site and major epitopes for neutralizing antibodies. The fibrous stem possesses the NH2 terminus of HA2 required for uncoating and infectivity (see Nayak, 1978, 1997, 2000, 2011).

NA (also known as sialidase, EC 3.2.18) is an exoglycosidase that cleaves the SA (*N*-acetylneuraminic acid) from glycans removing the SA receptor. NA is a type II membrane protein possessing a single extended hydrophobic domain at the N-terminus for both protein translocation across the ER and anchoring into lipid bilayer. Tetrameric NA spike exhibits a mushroomlike oblong head attached to a fibrous stalk. Each monomeric head consists of six propeller-like four-stranded antiparallel β sheet in a counterclockwise order containing enzymatic catalytic site in a large depression on the top of the

head. Therefore, each NA spike possesses four catalytic sites 40°A apart (Nayak, 1997). NA also contains four to six N-linked oligosaccharides, which vary for different NA subtypes. In type C virus, the HE protein provides both HA and NA functions. NA facilitates virus release from infected cells and plays an important role in virus spread, virulence, and host specificity. Antibodies against NA do not directly neutralize virus particles but significantly reduce virus pathogenicity by restraining virus spread within the host and the population. Antiviral agents like oseltamivir (Tamiflu; Roche) and zanamivir (Relenza; GlaxoSmithKline) interfere with NA function.

In type B virus, the NA mRNA segment also encodes another protein, NB of 100 aa, present only in the virus-infected cells but not in virus particles and has no known function.

M2, a small protein of 97 aa, is a homotetramer and functions as a proton channel. M2 is a TM type II protein with a single signal/anchor domain but unlike NA, its N-terminus is out and C-terminus in. M2 is encoded by a spliced mRNA of the segment seven RNA. Although only a few molecules (16–20/virion) of M2 are present on the virus envelope, it functions as a proton channel, and plays a critical role in the uncoating process during infection (Section Infectious Cycle). M2 affects host range, virus budding, and is critical for the function of antiviral agents (amantadine, rimantadine).

M1, the most abundant protein in virions, is present as the electron dense layer underneath the viral membrane forming the critical bridge between the viral envelope and the RNP core. M1 interacts with the cytoplasmic tails of HA, NA, and M2 on the outer side and the vRNA and NP on the inner side. M1 is a lipid-binding protein and plays critical roles in many stages of the virus replication including regulation of uncoating/disassembly, nuclear import and export of viral RNPs, transcription and replication of viral genome, and finally, transport and assembly of RNPs and virus morphogenesis and budding.

Internal Core

The core of IV particle is called nucleocapsid or ribonucleoprotein (Figures 1, 3(b), and 3(c)). The virus core consists of helical RNP segments containing negative-stranded vRNAs (eight separate RNA segments in influenza A and B and seven RNA segments in IV C) and NP along with minor amounts of the nuclear export protein (NEP) and 3P complex. Influenza RNA segments range approximately from 0.8 to 2.3 kb, the largest ones encoding the polymerase proteins (PB1, PB2, PA) and the smallest one encoding the nonstructural (NS1 and NS2/NEP) proteins. All viral RNA segments possess similar sequences at their 5′ as well as the 3′ termini, which are partially complementary to each other forming a partially double-stranded panhandle stem that functions in regulating transcription and replication.

The vRNA in RNP is wrapped around the NP scaffold and exposed outside for gliding interaction with the 3P complex during transcription/replication. In virus particles, the 3P complexes are present only at one end of the RNP and requires primers for transcription initiation indicating that only transcriptionally inactive RNPs are exported out of nucleus and packaged into virions. Purified RNPs possess a twisted rodlike structure that is folded back and coiled on itself and are about 13 nm in diameter but vary in length up to 120 nm, depending on the size of the vRNA segments (Noda and Kawaoka, 2010; Tao and Zheng, 2012). NP is a basic protein providing the scaffolding function for RNA template during transcription and replication. Active synthesis of NP is required for the replication of the viral RNA but not for the transcription of mRNA.

The 3P complex (also called the transcriptase, replicase, or RDRP (RNA dependent RNA polymerase)) consists of three proteins, PB1, PB2, and PA. They are the three largest viral proteins (approximately 750 aa each) and encoded by the three largest viral RNA segments. 3P complex functions both in the transcription and replication of the viral RNAs. Only about 15–20 molecules of 3P complex are present in infectious virus particles. Both the transcription and replication of IV RNA occur inside the nucleus of the infected cells. Polymerase proteins possess nuclear localization signals and are actively transported into the nucleus through nuclear pores.

Nonstructural Proteins

Two NS proteins (NS1 and NS2/NEP) encoded by the smallest RNA segment (segment #8), are found in the virus-infected cells. NS1 is transported into the nucleus and implicated in inhibiting host mRNA synthesis, nuclear export of viral mRNAs and translation of viral proteins but not found in virus particles. NS1 plays a critical role in virus virulence by counteracting the function of host interferons. However, NS2 is a misnomer since it is found in virus particle. Now, NS2 is appropriately called NEP, which plays a critical role in nuclear exit of viral RNP into cytoplasm and eventual transport to the budding site. On the other hand, NB protein, encoded by segment six RNA (which encodes NA) in influenza B virus-infected cells, is not found in IV particles and therefore classified as an NS protein.

Similarly, PB1-f2, a small 87 aa protein, is expressed from PB1 mRNA in a + 1 alternative reading frame and causes apoptosis of macrophages. It is present in many but not all influenza A virus strains. However, its role in virulence and pathogenesis in influenza epidemics and pandemics remains unclear. Influenza A virus genome can encode up to 14 proteins (HA, NA, NP, M1, M2, M42, NS1, NS/NEP, PA, PA-X, PB-1, PB-2, PB1–N40, and PB2), although all 14 proteins are not encoded in the RNAs of all IVs.

Infectious Cycle

Infectious cycle involves attachment (adsorption), entry, fusion, and uncoating; transcription and replication of viral RNAs; translation of viral proteins; transport, assembly of viral proteins and vRNPs; and budding of virus particles (**Figure 4**).

Attachment/Entry

The first step in the virus life cycle is virus attachment, mediated by the interaction of ligand (HA) with the cell surface receptor (SA). Both the cell surface SA and the receptor-binding site on HA vary among different virus strains and the varying affinity of HA to SA receptors plays a critical role in species specificity, zoonoses, pathogenesis, transmission, and pandemic behavior of IV strains. After cell surface binding, virions enter the cell via receptor-mediated endocytosis. Majority of IVs (~70%) use clathrin-coated pit (CCP) and the remainder (~30%) use

clathrin-independent vesicles for entry. Both pathways lead to viral fusion with endosomal membranes with similar efficiency. However, IVs do not use preexisting CCP but form *de novo* CCP at the attachment site. How virus binding forms *de novo* CCP remains unclear but most likely local membrane curvature caused by multivalent binding of spherical virus particle on the cell surface attracts the Bar domain of amphiphysin to promote CCP formation. Filamentous viruses enter the cell via micropinocytosis using nonclathrin, noncaveola, dynamin-independant endocytosis.

NA coevolves with the receptor specificity of HA and, although NA is not required for virus attachment and entry, it facilitates infection of ciliated respiratory epithelium *in vivo* by releasing virus particles from mucin, rich in SA, covering the respiratory mucosa. Virus entry is a dynamic process involving both attachment and elution and NA affects the dynamics of the entry process by causing virus elution. Once the virus particle gets inside the endosome, NA has little role in entry.

Disassembly

Following attachment/entry, virions must undergo the uncoating/disassembly in the acidic environment of endosome (~pH 5), which can be separated into two major steps: (1) fusion of the viral and endosomal membranes and (2) release of the RNP. Steps in the IV life cycle are schematically shown in Figure 4.

Fusion Process

When vesicles are held together in close proximity (~15 Å) local destabilization occurs mixing the membrane lipids causing fusion, and forming a single vesicle. However, when IV binds to the cell surface SA via HA and is endocytosed, the distance (~150 Å) between the virus and the endosomal membranes is far too great for fusion. HA being the major barrier must undergo conformational change for fusion to occur. For this, two conditions are necessary: HA (HA0) must be cleaved into HA1 and HA2, and be present in an acidic environment (~pH 5). HA cleavage generates the glycine-rich hydrophobic fusion peptide (20–24 aa) at the N-terminus of HA2, which at neutral pH remains buried in the native molecule about 100 Å from the globular head. Acidic pH of endosome converts HA into a metastable state causing the fusion peptide to project upward and insert into the target (endosomal) membrane. Still, the distance between the endosomal and viral membranes remains around 100 Å. Finally, the stem of HA spike splays apart bringing the viral and endosomal lipid bilayers close to each other for fusion, leading to the formation of hemifusion state in which outer layer of the lipid bilayers undergoes mixing without the mixing of the inner content. Subsequently, both layers of the lipid bilayers fuse and a pore is formed mixing the content of both vesicles. Six or more HA trimers are required for the formation of the fusion pore. Furthermore, both the fusion peptide at the N-terminus and the C-terminus TMD of HA2 directly interact with each other. The glycine ridge on the N-terminal arm of the fusion peptide is important for helix–helix interaction with the TMD. Because of the low pH-induced structural changes in HA, the fusion process is irreversible. Low pH treatment weakens M1-membrane bonding and renders the viral envelope pliable facilitating fusion (see Nayak et al., 2013).

Release of Viral RNP

Disruptions of the M1-RNP and M1-HA, M1-NA, M1-M2 interactions in acidic pH release the RNP into cytoplasm for its transport into the nucleus. The proton channel M2, which remains closed at neutral pH, opens up in acidic pH of the endosome acidifying the virion-interior and releasing vRNP into the cytoplasm. M1-free RNPs are then transported into the host nucleus through the nuclear pore complex for the transcription and replication of vRNA. Membrane fusion and RNP release in acidic pH are highly coordinated otherwise RNPs becomes trapped and not released in the cytoplasm. Blocking the M2 ion channel by drugs like amantadine or rimantadine increases the endosomal pH preventing virus uncoating and virus replication.

Transcription, Translation, and Replication

Following disassembly, the RNP–3P complex is transported into the nucleus of the infected cells through the nuclear pore with the help of the nuclear localization signals present in polymerase and NP proteins. Three types of RNAs are found in IV-infected cells: vRNA (− strand viral RNA), cRNA (complete + strand RNA used as template for vRNA synthesis, mRNA (+ strand) used for the translation of virus-encoded proteins). In the nucleus, the first event is the transcription of the plus strand viral mRNAs by the 3P complex from the minus (−) strand vRNA template (primary transcription). However, IV transcription is a unique process since the 3P polymerase cannot initiate *de novo* transcription and requires primers for transcription initiation. 3P complex snatches the 5′cap of the host pre-mRNAs in the nucleus and uses them as primers for viral mRNA transcription. PB2 of the 3P complex binds to the 5′cap of host pre-mRNAs and the endonuclease activity of the PA cleaves the 5′RNA cap for initiating viral transcription. Therefore, all viral mRNAs contain at the 5′ end a capped oligonucleotide of 10–13 bases derived from the host mRNAs, followed by the body of the mRNA, which is complementary from the second nucleotide of vRNA but lacks the last 17–22 nucleotides and possesses poly(A) track at its 3′ end (Nayak, 1997).

Unlike mRNA transcription, vRNA replication involves synthesis of the complete (full length) + strand RNA (cRNA) without either the capped host mRNA primer at its 5′ end or the poly (A) track at its 3′ end. The cRNA (+ strand) is then used as a template for vRNA (− strand) synthesis. The switch from mRNA to full-length cRNA synthesis requires continued synthesis of NP. Thus NP provides both the scaffolding function for presenting the template RNA to the 3P complex and the antitermination function for full length RNA chain completion. vRNA synthesis is also coupled with NP binding to the nascent vRNA into RNP.

Translation of viral proteins from mRNAs continues at a high rate throughout the infectious cycle, although the mRNA synthesis is greatly diminished in the late phase of infection. 3P proteins, Ml, NS1, NS2, and NP are synthesized on the free cytoplasmic polysomes, whereas TM proteins like HA, NA, M2, and NB are synthesized on membrane-bound polysomes. These TM proteins follow the same trafficking pathway as the host TM proteins and are glycosylated during transport. HA, NA, and M2 form complexes (trimer for HA or tetramer for NA

and M2) in the ER membrane, and are exported to the Golgi complex and the plasma membrane.

When IVs are passaged at a high multiplicity of infection, defective interfering (DI) virus (also called von Magnus) particles are generated. These DI particles contain shorter vRNA (DI RNAs), which are generated primarily from the internal deletion of the three largest vRNA segments encoding the 3P proteins (Nayak et al., 1985). These DI particles are not infectious and need the helper function of infectious particles for replication. Therefore, DI particles can replicate only in cells coinfected with infectious particles, but in turn, interfere and become the predominant particles at the expense of infectious particles.

Budding

IV particles are released into the environment by budding from the cellular membrane of nonpolarized cells or apical plasma membrane of polarized epithelial cells. Complete virus particles are not found inside the infected cells. Therefore, all virus components are brought to the budding site for assembly, bud initiation, bud maturation, and release (Figures 4(b) and 4(c)). However, these steps are rather continuum without strong stop/start checkpoints, i.e., completion of the previous step is not required for the initiation of the next step. Assembly of viral components into subviral complexes can occur during either transport or budding; bud initiation can occur before all components are brought to the budding site; even bud release can occur in the absence of complete set of RNPs or without any RNP (**Figure 3(b), (iii) and (iv)**).

For assembly and budding, all three subviral components, namely, the viral envelope (with HA, NA, and M2), M1, and RNPs are brought to the budding site, i.e., the apical plasma membrane in polarized epithelial cells. However, what determines the apical membrane, as the budding site, remains unclear. Although IV particles possess three TM envelope proteins (HA, NA, and M2) with apical determinants, they are not sufficient in selecting the apical budding site. Other viral components, particularly NP/RNP, may play an important role in selecting the budding site (Nayak et al., 2004, 2009, 2013). Since RNPs are synthesized in the nucleus they must be exported and brought to the budding site. M1 and NEP mediate nuclear export of RNP by forming the daisy-chain complex of (Crm1 and RanGTP)-NEP-M1-RNP. Furthermore, since the 3P complexes are found only at one end of the RNP inside virus particles, inhibition of transcription initiation of RNPs by M1 is critical for both nuclear exit and incorporation of RNPs into virus particles. The budding site is modified by the accumulation of HA and NA spikes, which exclude majority of the host cell membrane proteins (patching); subsequently, M1 proteins are attracted to the inner layer of the membrane and viral RNPs accumulate underneath the M1 protein at the assembly site; finally, virus particles will bud from the plasma membrane (Figures 4(b) and 4(c)).

Infectious IV contains eight (seven for influenza C virus) specific vRNA segments. However, how these multiple RNP segments are correctly assembled into virus particles remains unclear. Two models, 'random packaging' and 'specific packaging,' have been proposed. The 'random packaging' model predicts the presence of common structural elements in all vRNAs causing them to be incorporated randomly into virions and the incorporation of each RNP in released particles will depend on vRNA/RNP concentrations in the infected cells. Observations that some IV particles possess more than eight RNPs (9–11 RNAs per virion) and that at most 1 in 10 virus particles are infectious support random packaging. On the other hand, 'specific packaging' model predicts that specific structural features are present in each RNA/RNP segment enabling them to be selectively incorporated into virions. Support for this model includes (1) vRNAs are equimolar in virus particles, although vRNA concentrations vary in infected cells; (2) DI RNAs competitively inhibit packaging of their normal counterparts but not other vRNAs (Nayak et al., 1985); (3) recent studies from a number of laboratories have shown the presence of segment-specific packaging signal(s) in all eight RNA segments and incorporation of specific RNA segments supports a hierarchy RNA incorporation; (4) serial sectioning of influenza A virus infected MDCK (Madin-Darby Canine Kidney) cells as well as ET analysis of released virions showed that the RNPs of influenza A viruses are organized in a distinct 7 + 1 pattern (Noda and Kawaoka, 2010); and (5) FISH (Fluorescence In situ Hybridization) analysis demonstrated the presence of eight unique RNA segments in the majority of RNP-containing virus particles (Chou et al., 2012). However, specific RNA–RNA interactions forming multisegmental RNP complexes in trans are yet to be shown and moreover, IV particles without RNP are seen (**Figure 3(b), (iii) and (iv)**). It is, however, possible that segment-specific complex formation and incorporation of vRNA complexes can occur but do not affect bud release, i.e., RNPs are not active participants in the budding process but passively entrapped in the bud and released as virus particles.

Pandemics and Epidemics

Flu (or influenza) outbreaks occur almost predictably as epidemics in fall and end of spring. Although the severity of outbreaks varies from year to year, they are responsible for increased morbidity and mortality and economic loss in billions of dollars during that period. Since IV cannot survive outside the host for a long time and since the virus does not produce latent or chronic infections in patients, IV persists by spreading from person to person, evading the host immune defense. In winter months, close person-to-person contact because of indoor living and increased viability of IV in the dry cold air facilitate the spread of the disease among the population. Two unique properties of the IV are at the root of its epidemic behavior.

Antigenic Drift

High frequency of antigenic mutations (one per 10^5–10^6 nucleotides per infectious cycle) caused by the error-prone nature of the IV replicase, inefficient proofreading function and selection of resistant viruses by the host immune response lead to antigenic drifts and annual epidemics.

Epidemics are defined as geographically limited outbreaks of the disease in human population. Analyses of virus isolates from epidemics have shown that new mutant viruses continue to emerge almost every year. Although mutations occur in all eight viral RNA segments, selection pressure by the host

antibodies leads to the emergence of resistant antigenic variants. There are five major antigenic epitopes on the globular head of HA, which are altered in the new variant viruses to escape the host immune response elicited by prior infection (Nayak, 1997). Since some of the minor antigenic determinants may not be altered and since majority of the human population are likely to have antibodies against the progenitor virus, annual epidemics produced by the antigenic variants tend to be milder and are less widespread compared to the worldwide pandemics. Still, influenza is responsible for over 41 000 deaths every year during fall and winter seasons in the United States alone, particularly among the elderly people. It also causes economic impact as lost workdays, decreased work efficiency, and increased school absenteeism, hospitalization, clinic visits, and use of medication. For example, recent estimates put the cost of influenza epidemics to the economy of the United States at $71–167 billion per year (Molinari et al., 2007; http://www.who.int/mediacentre/factsheets/2003/fs211/en/).

Antigenic Shift

The segmented nature of the viral genome and a large gene pool of influenza A viruses (18 HA and 11 NA subtypes in humans as well as in other warm-blooded animals and birds) facilitate mixing of human and nonhuman viral genes and provide an unusual opportunity for exchanging viral genes by reassortment causing antigenic shift leading to new antigenic subtypes. Such antigenic shifts are responsible for worldwide pandemics, which unlike seasonal epidemics, are global in nature, spreading rapidly among the human population. Jet age mobility further aids in the rapid spread of the virus. In the last century, three influenza pandemics were documented. Spanish flu pandemic of 1918, the deadliest pandemic in human history, killed over 20 million people worldwide including 500 000 in the United States alone. Subsequently, two other pandemics (Asian flu or Japan flu in 1957 and Hong Kong flu in 1968) occurred in the twentieth century. The nature of the 1918 virus was determined later from the antibodies in the sera of the people afflicted in that year since no virus was available. However, recently, the 1918 virus genome was sequenced from tissues preserved in formalin or frozen in permafrost and the 1918 virus was recreated by reverse genetics (Tumpey et al., 2005). The first pandemic of the twenty-first century was declared by WHO in 2009. Analyses of pandemic viruses show that all pandemics are caused exclusively by type A viruses. An H1N1 swine influenza-like virus was responsible for both 1918 and 2009 pandemics, H2N2 virus for 1957, and H3N2 virus for 1968 pandemics. The 2009 pandemic virus (A/Cal/04/2009) (H1N1) (also known as swine flu) is derived by mutations and quadruple reassortment of swine, avian, and human IVs (Neumann et al., 2009; **Figure 5**). However, 2009 H1N1 virus is antigenically distinct from previous H1N1 viruses.

Although IVs are species specific (i.e., they produce disease and spread within the same host species but do not cause disease in other animal species), humans are known to become occasionally infected with animal IVs (e.g., farmers are found to have antibodies against swine, equine, or avian viruses) and vice versa. Therefore, occasionally, a person (or an animal) becomes infected at the same time by multiple viruses, e.g., a human and an animal virus, and both viruses replicate in the same cell. Segmented nature of the viral genome facilitates gene reassortment during the assembly and packaging of viral RNPs into the virus buds causing emergence of new viruses containing RNA segments from different viruses. Some of the new viruses will retain the growth advantage for humans while replacing the antigenic determinants of old HA or both HA and NA with those of the animal viruses. Further mutations and reassortment facilitate emergence of a new pandemic virus. The most recent 2009 H1N1 pandemic virus emerged from multiple reassortments and mutations (**Figure 5**). However, conditions leading to the advent of new pandemics are not fully understood, making it impossible to predict either the time or the nature of the next pandemic virus. Recent avian viruses (H5N1 and H7N9) that can cause high mortality in infected humans but do not spread from human to human are of great concern to WHO.

Genetic reassortment between two or more viruses is routinely used for making current influenza vaccines in chicken embryos. Each year the newly isolated candidate virus that is predicted by WHO to cause epidemic next year, is grown together with a high-yielding A/PR/8 virus to generate the high-yielding vaccine virus that will contain the HA and the NA of the new strain.

Disease

Typical influenza is an acute illness often characterized by sudden onset of fever, myalgia, sore throat, and nonproductive cough. However, clinical manifestations of influenza infection vary greatly from person to person. Many host factors such as age, immunological status (i.e., prior exposure to antigenically related viruses), physiological state of the infected person and many other factors influence severity of the disease syndrome. Infection may be subclinical (i.e., asymptomatic), or produce mild symptoms like fever or cold, or cause an acute prostrating febrile illness accompanied by general and respiratory symptoms. These may include pharyngitis, tracheitis, bronchitis leading to shortness of breath, cough, and myalgia. In severe cases, it may lead to primary viral pneumonia accompanied with subbronchial hemorrhage and even fatal outcome. Incubation period is short (1–2 days); onset is sudden with fever (temperature up to 102 °F or even higher), chill, and myalgia. In young children, common symptoms are pharyngotracheobronchitis, croup, and even gastrointestinal disorder. In a typical flu in adult, fever may subside in 3 days but respiratory symptoms and general weakness may persist for 2–3 weeks. The disease may be severe in very young or elderly patients or in patients with other preexisting underlying conditions including pregnancy, cardiovascular diseases, respiratory conditions such as chronic bronchitis (chronic obstructive pulmonary disease), bronchopulmonary neoplasia, asthma, emphysema, as well as in patients with immunocompromised conditions including radiation and chemotherapy (Nayak, 1997).

The virus is commonly spread by aerosol droplets and by fingertips. Therefore, washing of hands and avoiding contact with infected persons are the first lines of defense against the

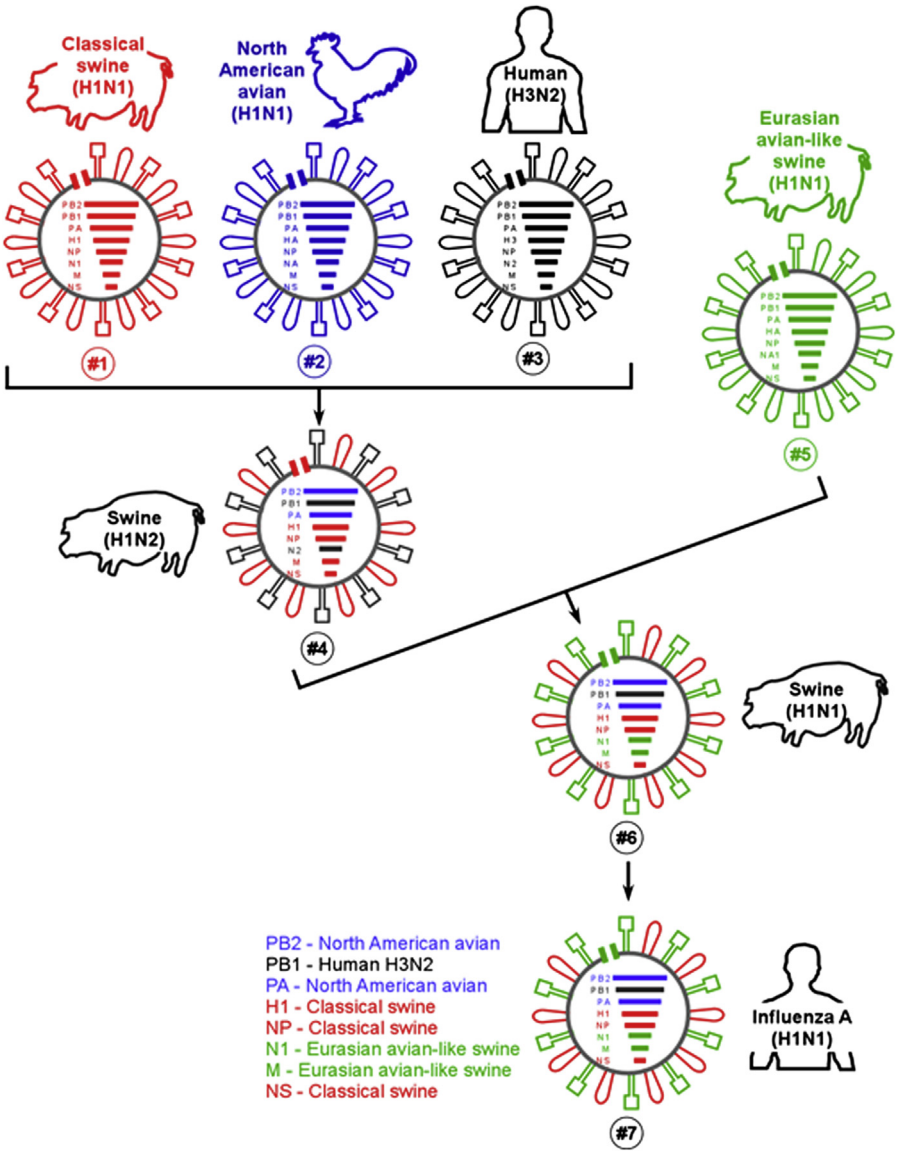

Figure 5 Genesis of H1N1 2009 pandemic virus of swine origin (A/Calif/04/2009(H1N1)). A new pandemic human virus (H1N1, #7) emerged from mutations and quadruple reassortment of swine, avian, and human viruses. First, a swine virus (H1N2, #4) emerged from triple reassortment of classical swine (H1N1, #1), North American avian (H1N1, #2) and human (H3N2, #3) viruses. This virus (H1N2, #4) circulated in North American swine population and further reassorted with Eurassian avianlike swine virus (H1N1, #5), generating swine (H1N1, #6) virus that emerged as a new pandemic human virus (H1N1, #7) of swine origin in 2009. Adapted from Neumann, G., Noda, T., Kawaoka, Y., 2009. Emergence and pandemic potential of H1N1 Influenza Virus. Nature 459, 931–939.

spread of flu. Although indoor living and closeness of people in an enclosed environment during the winter season facilitate virus transmission from person to person leading to epidemics, there is no clear correlation between the severity of the winter and either the frequency and severity of the clinical disease or epidemics.

Pathogenesis and Virulence

IV is lytic and usually does not establish a chronic or stable relationship in infected cells/hosts. It causes an acute illness by killing the infected cells. Virulence and pathogenesis of IV depends on its ability to grow and kill cells in a particular tissue, namely, respiratory tract, leading to reduced functional ability of the organ. Cell killing by IV involves (1) cleavage and degradation of the newly synthesized cellular mRNA, 5′cap of which is used as the primer for viral mRNAs; (2) suppression of the synthesis of the cellular proteins; (3) modification of the plasma membrane that may contribute toward *in vivo* killing of virus-infected cells by host immune response (cytotoxic T cells); (4) apoptosis of infected cells, which can be induced by a number of virus factors including dsRNA, NS1, NA, and PB1F2. Multiple apoptosis inducing pathways including tumor

necrosis factor-related apoptosis inducing ligand are involved in cell death.

Clinical symptoms, including the severity of the disease, are related to the extent of desquamation of respiratory epithelial cells and the site of destruction (i.e., upper respiratory, middle or lower respiratory tract). Experimental studies in animals suggest that influenza is a descending infection, i.e., the virus initially infects either the upper respiratory or the tracheal epithelium and later the infection spreads to bronchial epithelium or even to alveolar cells causing primary viral pneumonia. In general, severity of the disease depends on the ability of the virus to infect and spread in the lower respiratory epithelium including bronchiolar and alveolar cells. On the other hand, spread among the population depends on the ability of virus to replicate in nasal and upper respiratory epithelium. Therefore, avian virus like H5N1 in recent years, although highly virulent in infected persons (>50% fatality), does not spread among humans. A virus that grows efficiently in both upper and lower respiratory tracts can cause severe disease and spread efficiently. Nature of the SA receptor on the upper and lower respiratory cells can vary and the differential binding efficiency of the virus HA to SA receptors can affect both virulence and spread of the virus among the population. Usually, the virus is shed for about a week, and sometimes longer in rare cases with persistent pulmonary disease or in immunocompromised patients.

Although respiratory symptoms can be explained on the basis of the site and extent of viral pathology, the cause of general symptoms such as myalgia, anorexia, malaise, or gastrointestinal disorder remains unexplained, especially since influenza infection usually does not cause viremia (virus in blood) and spread to other organs. A number of unusual symptoms such as myopathy, myocarditis, and encephalopathy have been occasionally associated with influenza, again, without a rational explanation.

Although in an epidemic, the variation in the disease syndrome from one person to another is primarily due to host factors (since the same virus strain infects all persons), viral genes affecting virulence are critically responsible for the overall nature and severity of the epidemics/pandemics. Basically, viral genes that regulate tissue tropism, replication efficiency, and cell killing ability, also determine the virulence of a virus strain. IV virulence is multigenic (i.e., determined by several viral genes). For example, HA is a critical virulence factor because of its variation in binding efficiency to SA, cleavage efficiency, and antigenic epitopes. The cleavage of HA by a host protease is critical in producing infectious virus; the length and the nature of the amino acids of the connecting peptide (including the presence of multiple basic amino aa, e.g., arginine, lysine) between HA1 and HA2 affect virulence. NA is also an important determinant for spreading of virus and therefore in virulence. Furthermore, each of the three P proteins, and NP, M1, M2, and NS1 also affect virulence.

Although all pandemics and most epidemics are caused by influenza A viruses, influenza B epidemics are not uncommon. Influenza B viruses are more common in children than in young adults. Both influenza A and B viruses produce similar symptoms, although influenza B infections are usually milder than influenza A infections. However, influenza B virus can also cause severe and even fatal illness. Infections by influenza C virus are less common, and produce milder symptoms than by either A or B viruses.

Host Response

The progression and outcome of a disease in humans is a complicated process and based not only on the viral virulence but also on the host response. Following virus infection, initial host response is the production of interferons, which are likely to slow the process of virus replication. NS1 protein of IV is a potent tool to counteract host interferons. Subsequently, induction of three major classes of antibodies (IgM, IgG, and IgA), and cytotoxic T cells facilitate limiting the disease, and promoting recovery. IgM is produced after primary infection, and subsequent response is limited to IgG and IgA. Although antibodies are generated against all viral components, antibodies against HA and NA provide protection and limit virus spreading. After recovery, the person becomes solidly immune against reinfection by the same IV. Secretary IgA in the nasal and respiratory mucosa is effective in preventing reinfection. Humoral IgG against HA provides the neutralizing antibodies and is easily assayed by hemagglutination inhibition (HI). Antibodies against NA do not neutralize virus but prevents virus spreading and infection of new cells/hosts. Cytotoxic T cells are generated against both the surface (HA) and internal viral components (particularly NP).

Complications

The most frequent cause of complications is secondary bacterial infections resulting in bacterial pneumonia. Pneumococci, *Haemophilus influenza*, and staphylococci are most frequently isolated from fatal cases of influenza. The onset of bacterial infection usually follows after the symptoms of viral infections have subsided and is accompanied by the return of sudden chill, fever, productive cough, chest pain, and shortness of breath. These bacterial complications are more common in patients with preexisting lung conditions or in immunocompromised patients. The pneumonia due to secondary bacterial complication essentially follows the same course as the disease caused by the bacteria alone and appropriate antibiotics are effective against bacterial infections.

Other uncommon complications are encephalitis, myocarditis, and postinfection neuritis that is indistinguishable from the Guillain–Barre syndrome. Another serious and often fatal complication called Reye's syndrome has been implicated with influenza B virus (rarely with influenza A virus) and varicella (chicken pox) virus along with aspirin (salicylates) medication. It is an acute noninflammatory encephalopathy, cerebral edema accompanied with fatty degeneration of liver. The onset of the disease occurs within 4–7 days of infection with repeated vomiting, disorientation, delirium, and coma.

Diagnosis

Diagnosis based on the clinical symptoms alone cannot definitely differentiate IV infections from other similar flulike

conditions unless in an ongoing epidemic. Many other viruses such as respiratory syncytial virus, adenoviruses, coronaviruses, rhinoviruses, parainfluenzaviruses, or even human immunodeficiency virus cause 'flulike' symptoms. Definitive virological diagnosis is done only in the laboratory. For rapid laboratory diagnosis, specimens are collected by swabbing the posterior pharynx; viral antigen can be detected in the nasopharyngeal cells by staining with antibodies conjugated to an immunefluorescence dye. Swab specimens can be used for growing virus in appropriate cell culture (MDCK cells) or chicken embryos, in which virus can be detected by immunefluorescence, hemadsorption, or hemagglutination. Specific antibodies against internal antigens (NP and Ml) are used for determining the virus type (i.e., type A, B, C) and antibodies against HA and NA for subtype specificity. Recently, newer diagnostic assays such as ELISA, PCR, genome sequencing, and other immunological and molecular biological tools are used. Usually, virological diagnosis is carried out by the Public Health Laboratories and is important in determining the cause and the nature of the epidemic.

Treatment

Current antiinfluenza drugs are limited to two families and are based on either interfering with the process of uncoating and RNP-release by blocking the M2 proton channel (amantadine and rimantadine) or preventing the spread of postbud virus particles by inhibiting the enzymatic activity of NA (oseltamivir or Tamiflu by Roche and zanamivir or Relanza by GlaxoSmithKline). M2 inhibitors are effective only against influenza A and NA inhibitors are effective against both influenza A and B viruses. These drugs have been shown to be beneficial in reducing the intensity of the disease, particularly if administered early in infection and can be used as chemoprophylactic agents during an ongoing influenza A epidemic in high-risk groups in nursing home environment. However, chemoprophylaxis is not a substitute for vaccination and is recommended only for short-term prophylaxis for later vaccination of high-risk persons. Some of the side effects of the drugs are headache, confusion, insomnia, and slurred speech. In addition, antiviral treatment leads to the emergence of drug-resistant virus strains.

Usually influenza is a short illness with uneventful recovery. Therefore, general supportive treatment is recommended in most cases. These include rest, light diet, and avoiding dehydration. Other symptomatic treatment to reduce fever, or suppress cough may be given as required. In secondary bacterial pneumonia, appropriate antibiotics should be used. In addition, in viral or bacterial pneumonia, oxygen therapy may be needed to reduce hypoxemia.

Prophylaxis

Because influenza infection leads to a solid immunity against the same virus, many attempts have been made to produce a safe and effective vaccine against flu epidemics. Currently, WHO and CDC recommend two types of vaccines. (1) One is inactivated whole virus vaccine or a subunit vaccine (also called split virus) containing components of the viral envelope (HA and NA). For previously unvaccinated children, subunit vaccines should be administrated twice, 1 month apart, intramuscularly. For adults and children vaccinated before, one injection is recommended. Vaccine should be given annually before the flu season (i.e., early fall) because the immunity declines in the year following vaccination. (2) The other is live attenuated vaccine using cold adapted virus, which is given as a nasal spray. Both are effective 70–90% in the population. Protection depends on the age and immune competence of the vaccine recipients and the similarity between the vaccine virus and the circulating virus. With a good match, vaccine prevents illness in 70% of the vaccinated adults (65 years old). Among the elderly persons in nursing homes, the vaccine is 50–60% effective in preventing mortality, even though it is only 30–40% effective in preventing influenza illness among frail elderly. Vaccine also plays an important role in reducing the spread of infection in nursing homes through the herd immunity. To be most effective, the vaccine virus should be of the same subtype as the epidemic virus. This poses a logistic problem because the vaccine viruses for the next fall are usually selected by the Public Health Authorities earlier in the spring. Epidemiological criteria of the virus variants in circulation are used to predict the next year's epidemic strains. The vaccine strain is then converted to a high-yielding virus for growing in chicken embryos by reassortment with high-yielding A/PR/8 virus. Currently, all vaccines whether live or split virus are produced in embryonated chicken eggs. Although epidemic virus may differ from the vaccine strain, vaccination elicits both the variant-specific antibodies against the vaccine virus and heterovariant (homosubtypic) antibodies against earlier IV infection. This phenomenon, called 'original antigenic sin' (Francis, 1960), is due to amnestic response resulting from the stimulation of the cross-reactive memory B cells. HI titer provides an indicator of protection against a specific subtype virus. To be more effective, the vaccine must produce antibodies against both HA and NA as obtained with the complete unmodified virus. Cytotoxic T-cell response is produced inefficiently by the inactivated or subunit vaccine but efficiently by live attenuated vaccine. Individuals with chronic diseases may develop lower antibody titer. Nevertheless, even in such individuals, vaccine is effective in preventing lower respiratory tract infection, reducing the risk of pneumonia, hospitalization, and death.

Antigenic drifts as well as short duration of the humoral immunity induced by subunit vaccine require annual vaccination. Vaccination with whole virus sometimes produces side effects including pain at the site of inoculation and febrile condition. Subunit vaccine reduces these toxic reactions greatly and is particularly recommended for children. During 1977 swine influenza campaign, Guillam–Barre syndrome (also known as radiculoneuritis or acute infective polyneuritis, temporary motor weakness or loss of reflex) was an unconfirmed complication of vaccination. Inactivated IV vaccine should not be given to persons with known anaphylactic hypersensitivity to eggs and to persons with acute fever until recovery.

In summary, the current vaccines elicit good immune response and are effective in reducing the morbidity and

mortality in the target groups. The recommended target groups by WHO are

1. residents of institutions for elderly people and the disabled;
2. elderly, noninstitutionalized individuals with chronic heart or lung diseases, metabolic or renal disease, or immune deficiencies;
3. all individuals >6 months of age with any of the conditions listed above;
4. elderly individuals above a nationally defined age limit, irrespective of other risk factors; and
5. other groups defined on the basis of national data and capacities, such as contacts of high-risk people, pregnant women, health care workers and others with key functions in society, as well as children 6–23 months of age.

Common tips in flu prevention are the following:

1. Stay home if you have flulike symptoms and for at least 24 h after fever has subsided.
2. Get vaccinated and talk with your health care provider.
3. Wash your hands for at least 20 s.
4. Cover your cough and sneezing with your elbow and not with your hands.
5. Clean your work area with disinfectant.

Conclusion

Influenza is a worldwide infectious viral disease of great public health importance both in the developed and developing countries causing increased morbidity and mortality in the population and economic impact in billions of dollars. A recent study (Molinari et al., 2007) estimated that in the United States alone, annual influenza epidemics result in approximately 600 000 life-years lost, 3 million hospitalized days, and 30 million outpatient visits, resulting in medical costs of $10 billion annually. According to this study, lost earnings due to illness and loss of life amounted to over $15 billion annually and the total economic burden of annual influenza epidemics amounts to over $80 billion. Also, in the United States, the flu season usually accounts for 200 000 hospitalizations and 41 000 deaths. Hospitalization and deaths mainly occur in high-risk groups (elderly, chronically ill). Worldwide, although difficult to assess, these annual epidemics are thought to result in between 3 and 5 million cases of severe illness and between 250 000 and 500 000 deaths every year ('WHO Influenza' http://www.who.int/mediacentre/factsheets/2003/fs211/en/). Most deaths currently associated with influenza in industrialized countries occur among the elderly over 65 years of age. Clearly, more effective drugs and better vaccines are needed to combat the impact of flu epidemics since the current antiinfluenza drugs are limited to two families, namely, proton channel blocker and NA inhibitors and viruses often become resistant to these drugs. A better understanding of the virus life cycle could provide us with newer viral and host targets for intervention leading to the development of newer therapeutic and prophylactic agents. Similarly, a better and universal vaccine eliciting long-lasting humoral and cellular immunity will reduce the worldwide impact of both annual epidemics and infrequent global pandemics.

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See also: Human Immunodeficiency Virus Type-1; Toga Viruses; Vaccines; Viral Pathogenesis.

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