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Pathogenicity and virulence of influenza

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ABSTRACT

Influenza viruses, including four major types (A, B, C, and D), can cause mild-to-severe and lethal diseases in humans and animals. Influenza viruses evolve rapidly through antigenic drift (mutation) and shift (reassortment of the segmented viral genome). New variants, strains, and subtypes have emerged frequently, causing epidemic, zoonotic, and pandemic infections, despite currently available vaccines and antiviral drugs. In recent years, avian influenza viruses, such as H5 and H7 subtypes, have caused hundreds to thousands of zoonotic infections in humans with high case fatality rates. The likelihood of these animal influenza viruses acquiring airborne transmission in humans through viral evolution poses great concern for the next pandemic. Severe influenza viral disease is caused by both direct viral cytopathic effects and exacerbated host immune response against high viral loads. Studies have identified various mutations in viral genes that increase viral replication and transmission, alter tissue tropism or species specificity, and evade antivirals or pre-existing immunity. Significant progress has also been made in identifying and characterizing the host components that mediate antiviral responses, pro-viral functions, or immunopathogenesis following influenza viral infections. This review summarizes the current knowledge on viral determinants of influenza virulence and pathogenicity, protective and immunopathogenic aspects of host innate and adaptive immune responses, and antiviral and pro-viral roles of host factors and cellular signalling pathways. Understanding the molecular mechanisms of viral virulence factors and virus-host interactions is critical for the development of preventive and therapeutic measures against influenza diseases.

1. Introduction

Influenza viruses (flu) have a global distribution and are both human and animal pathogens. Human influenza viruses cause annual epidemics (seasonal), which are highly contagious respiratory infections that can lead to severe illness and life-threatening complications in high-risk groups [1]. Occasionally, a new human influenza virus strain would arise from an animal origin and spread rapidly among human populations that have no pre-existing immunity, causing excessive mortality and morbidity globally, known as a pandemic. There have been four influenza pandemics in modern times, the 1918–19 "Spanish" flu, the 1957 "Asia" flu, the 1968 "Hong Kong" flu, and the 2009 "Swine flu." The 1918–19 "Spanish" flu was the most severe pandemic in recent history, which was estimated to have caused ∼ 500 million infections and 50–100 million deaths worldwide [2]. Humans can also be sporadically infected by animal influenza viruses (zoonotic), most often avian and swine flu; however, they have yet to establish sustained infection in humans. In particular, avian H5 and H7 influenza A viruses have caused hundreds to thousands of infections in humans, with

a high case fatality rate (30–50%) (reviewed in [3]). The likelihood of these avian influenza viruses gaining efficient human-to-human transmissions to cause the next pandemic poses a significant public health risk.

Current control measures for influenza infections include vaccines and antiviral drugs. However, influenza viruses have evolved rapidly to escape vaccine immunity and develop drug resistance. New viral variants exhibit reduced binding of vaccine-elicited neutralizing antibodies, causing vaccine mismatches or reduced vaccine efficacy. Mutations resistant to US Food and Drug Administration (FDA)-approved antiviral drugs have been detected [4]. Some become predominant in circulating strains, making the class of antivirals, such as M2 inhibitors, ineffective. Broadly protective vaccines and novel therapeutics are urgently needed to combat ever-changing influenza viruses.

Significant progress has been made in the understanding of the molecular mechanisms of influenza viral replication, transmission, and disease pathogenesis. This review summarizes the current knowledge of viral genetic markers and host responses affecting

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influenza virus pathogenicity and virulence, which will help in the development of better prevention and treatment options.

## 2. Mechanism of influenza virus replication and pathogenesis

2.1. Influenza virus genome and genes

Influenza viruses belong to the *Orthomyxoviridae* family and include four major types A, B, C, and D. Influenza A virus (IAV) can infect a wide range of avian and mammalian species, including humans, birds, ducks, chickens, turkeys, pigs, horses, and dogs [5]. Influenza B virus (IBV) infects humans and seals, whereas influenza C virus (ICV) infects humans and pigs [5]. IAV, IBV, and ICV are human pathogens. The influenza D virus (IDV), discovered in 2011, infects pigs and cattle, with no human infections reported to date [6]. This review focuses primarily on IAV as it causes most human and animal flu infections.

IAV are enveloped RNA viruses with eight single-stranded negative-sense RNA segments (Figure 1a). Viral envelope proteins include two major glycoproteins, haemagglutinin (HA) and neuraminidase (NA), and the transmembrane protein M2. Beneath the viral membrane is the matrix lattice formed by the M1 protein. Inside viral particles are eight viral RNA

(vRNA) segments in the form of the viral ribonucleo-protein complex (vRNP), of which each vRNA is encapsidated by nucleoproteins (NPs) and associated with the PB1/PB2/PA polymerase complex (Figure 1b).

Each vRNA segment encodes one to three proteins via alternative splicing or translation mechanisms (Figure 1c). There are a total of ten essential proteins for viral infection (PB1, PB2, PA, NP, HA, NA, M1, M2, NS1, and NEP/NS2) and several accessory proteins, such as PB1-F2 and PA-X, which mediate virus-host interactions, modulate innate immunity, and affect viral pathogenicity (reviewed in [7]). PB1 (containing an RNA-dependent RNA polymerase domain), PB2 (containing a cap-binding domain), and PA (containing an endonuclease domain) form a heterotrimeric polymerase complex that is essential for viral RNA synthesis. NP encapsidates vRNA and complementary RNA (cRNA) and is also required for viral RNA synthesis as demonstrated by influenza virus minigenome assay [8,9]. HA binds to the host receptor and mediates membrane fusion. NA facilitates the release and spread of progeny virions by cleaving sialic acid on the cell surface. M1 mediates viral particle assembly and budding from the plasma membrane. M2 is an ion channel protein on the viral membrane that is required for viral replication and modulation of cellular homoeostasis. NS1 impairs host antiviral responses via multiple mechanisms as described below in details. NEP/NS2
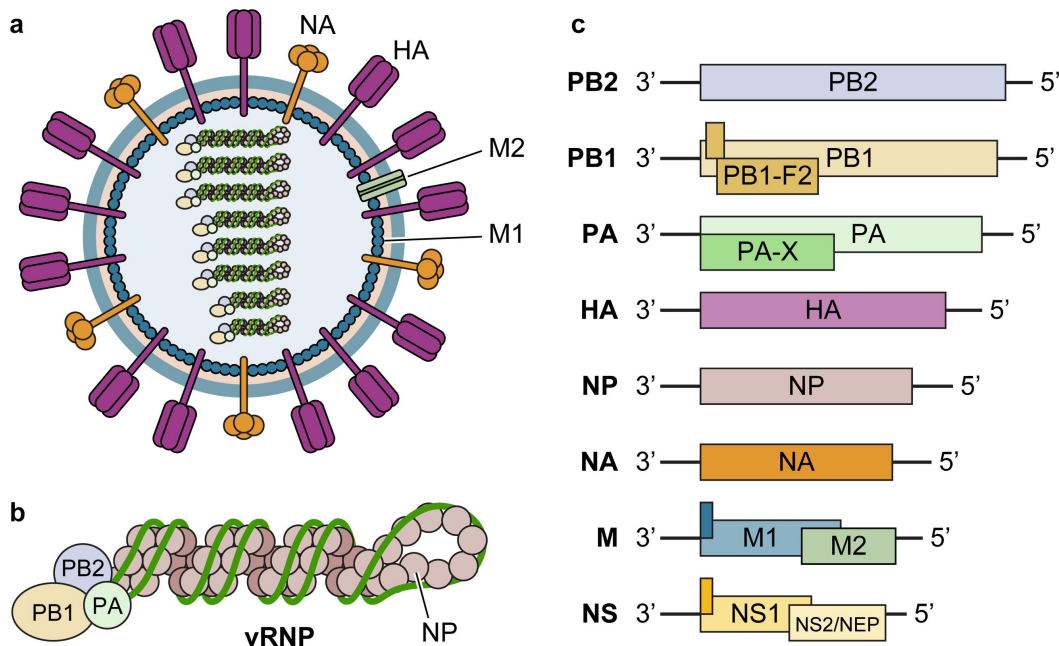
Figure 1. Illustration of influenza virion components, genomic organization, and viral genes. (a) Influenza virus is an enveloped RNA virus, which has 3 envelope proteins (HA, NA, and M2) on the viral membrane, an M1-formed matrix layer, and eight vRnps. (b) Each vRNP consists of one vRNA segment wrapped with NP and associated with polymerase complex PB2/PB1/PA. (c) Each vRNA segment encodes 1–3 genes, through alternative splicing (NS2/NEP and M2) and frameshifting (such as PA-X and PB1-F2). Accessory proteins expressed through frameshifting are shown as filled dark green bars/boxes.

facilitates the export of vRNAs from the nucleus to the cytoplasm for assembly.

2.2. Influenza virus life cycle

To initiate an infection, the influenza virus first binds to the cell surface receptor, sialic acid residues of glycoproteins or glycolipids, through the receptor-binding site (RBS) in the HA protein. Viruses are internalized into early endosomes and trafficked to late endosomes, where the low pH environment causes conformational changes in HA to expose a fusion peptide that leads to the fusion of viral and endosomal membranes. The M2 proton channels on the viral membrane mediate H^+ influx into the viral interior, which lowers the pH to facilitate the release of vRNPs from M1 into the cell cytoplasm in a process called uncoating [1,7].

The released vRNPs are then imported into the nucleus for viral RNA replication and transcription. Viral RNA synthesis is mediated by the heterotrimeric polymerase complex PB1/PB2/PA [10]. The polymerase complex recognizes the terminal promoter sequence of each vRNA segment and generates complementary positive-strand RNA (cRNA), which is then used to produce more copies of vRNA. mRNA is transcribed from vRNA and initiated by cap structures stolen from cellular pre-mRNAs in a cap-snatching mechanism, by which PB2 binds to the 5' m [7]G cap, and the PA endonuclease cleaves it. The 3'-polyadenylated tail is added to viral mRNAs through a stuttering mechanism. Thus, influenza mRNAs are 5-' capped and 3-polyadenylated. Some viral transcripts (M and NS) are processed by the host RNA splicing machinery. Both primary and processed viral transcripts are exported to the cytoplasm for translation by the host ribosomes. PB1, PB2, PA, and NP are imported into the nucleus to increase viral RNA synthesis and vRNP formation. Viral membrane proteins (HA, NA, and M2) traffic to the plasma membrane through cellular secretory pathways. Other non-structural proteins, such as NS1, PB1-F2, and PA-X, modulate host cell responses.

The newly synthesized vRNPs are exported from the nucleus with assistance from M1 and NEP/NS2, and transported to the plasma membrane for assembly and budding [11]. M1 interacts with both viral membrane proteins and vRNPs to mediate viral particle formation and is also a major player in virus budding. Eight vRNPs are packaged into viral particles in a "1 + 7" configuration, where one central vRNP is surrounded by 7 vRNPs [12]. The newly formed progeny virions form aggregates at the cell surface because of the binding of HA to sialic acids on viral envelope proteins and on the cell surface. NA neuraminidase activity is thus required to release new virions that can spread to infect new target cells.

Three classes of FDA-approved antiviral drugs target different stages of the viral life cycle [4]. The M2 inhibitors (M2I) – amantadine and rimantadine – block M2 ion channel activity. Zanamivir and oseltamivir are NA inhibitors (NAI) that prevent the release of viral particles from the cell surface and stop the viral spread. Baloxavir marboxil (BXM) is an inhibitor of PA endonuclease activity (PAI) and suppresses viral transcription. Drug-resistant mutations have been identified in each class of antivirals [4]. Resistance to M2Is develops rapidly and requires a single or a limited number of mutations in M2 such as L26F, V27A, and S31N, which are prevalent in circulating influenza viral strains [13]. Thus, the M2Is amantadine and rimantadine are no longer recommended to treat influenza infections. NAI-resistant mutations such as H275Y, E119G, I223R, R292K, and Q136K, as well as PAI-resistant mutations such as I38M or I38T, have been identified from influenza isolates [4,14,15], though their global frequency was still low [16]. Nevertheless, novel classes of antiviral drugs and/or host-based therapeutics should be developed to treat influenza infections.

2.3. Influenza virus pathogenesis

Human influenza viruses are transmitted through the respiratory route, whereas avian influenza viruses are spread between birds primarily through direct contact via faecal-oral, faecal-faecal, and faecal-respiratory routes [1]. Viruses replicate mainly in the epithelial cells lining the respiratory or intestinal tract [1,17]. Virus replication peaked at ~48 h after inoculation and declined slowly thereafter, with little shedding after 6–8 days [17]. Mild influenza infections generally involve the upper respiratory tract and trachea, whereas the most severe and fatal human infections are associated with viral infections in the lower respiratory tract. Influenza virus causes the death of epithelial cells through various mechanisms. In addition, infected epithelial cells release cytokines and chemokines to attract infiltrating inflammatory cells such as neutrophils and macrophages and activate adjacent endothelial cells. These activated immune and non-immune cells produce even more inflammatory cytokines, such as IL − 6, IL − 1β, TNFα, and CCL − 2, to stimulate further infiltration, damage the epithelial-endothelial barrier, and cause more epithelial cell death. Zoonotic avian H5N1 viruses can cause a highly lethal disease in humans, which is associated with viral dissemination to broad tissue tropism, high viral titers in multiple organs [18,19], and a hyperinflammatory response [18]. Thus, the pathogenesis of severe influenza disease is caused not only by direct viral cytopathic effects but also by

exacerbated host inflammatory responses [18,20]. Therefore, effective therapeutics should aim to reduce both viral replication and pathogenic airway inflammation.
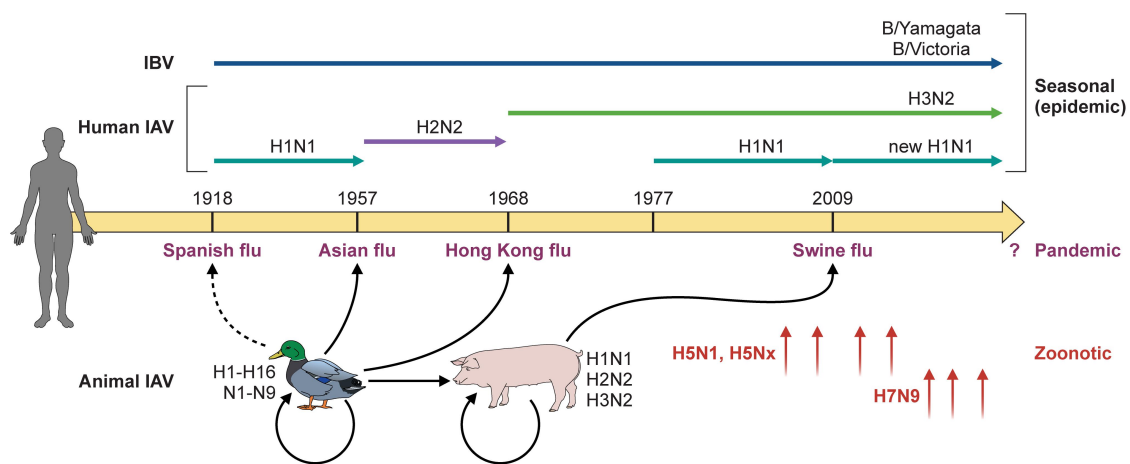
3. Mechanism of influenza virus evolution

3.1. History of influenza virus evolution

IAVs have 18 HA-and 11 NA-known subtypes. Except for H17N10 and H18N11 from bats, all other 16 HA and 9 NA subtypes were found in aquatic birds (ducks, geese, and shorebirds) [21]. Avian influenza viruses can infect poultry (chickens, turkeys, and domestic ducks), causing major economic damage. A few of these avian-origin subtypes have established within-host infections in other species, such as H1N1, H2N2, and H3N2 in humans and pigs; H7N7 and H3N8 in equines; and H3N8 and H3N2 in canines.

Human influenza viruses have changed over the years [4] (Figure 2). Since the 1918 Spanish pandemic flu, the IAV subtype H1N1 persisted in humans until it was replaced in 1957 by an H2N2 subtype (Asian pandemic). H2N2 circulated in humans until 1968 when it was replaced by a H3N2 subtype (Hong Kong pandemic). The H1N1 virus reappeared in 1977 and was replaced by a new H1N1 strain in 2009 (swine pandemic). Currently, two IAV subtypes, H1N1 and H3N2, together with two lineages of IBVs (B/Yamagata and B/Victoria) [22], co-circulate in humans. Therefore, annual flu vaccines are quadrivalent and contain two IAV subtypes and two IBV lineages.

Humans are occasionally infected by influenza viruses from pigs and birds, which sometimes results in severe disease and death, but has not been established in humans. Avian influenza viruses of subtypes H5, H6, H7, and H9 are known to cause zoonotic infections. In particular, H5 and H7 have caused major outbreaks in birds, with significant losses in the poultry industry and thousands of human infections with a high case fatality rate (CFR) (reviewed in [3]). The highly pathogenic avian influenza virus (HPAIV) H5N1 was first detected in Hong Kong in 1997 and has been circulating among domestic poultry and wild migratory birds in Asia, Europe, and Africa since 2003. The avian H5N1 virus has caused 868 laboratory-confirmed human infections and 457 deaths globally from 2003 to 2022 (CFR:53%) [23]. Novel H7N9 viruses that emerged in China in 2013 have caused a total of 1,568 laboratory-confirmed human infections, including 616 fatal cases (CFR:39%) as of January 2023, according to the WHO avian influenza weekly update [24]. These avian influenza viruses have yet to establish efficient human-to-human transmission, but their potential acquisition of airborne transmissibility through continuing viral evolution poses a potential pandemic threat [25]. Since the fall of 2022, an HPAIV H5N1 of clade 2.3.4.4b was found to infect many mammalian species, including foxes, cats, ferrets, seals, grizzly bears, and humans, and more alarmingly, spread among minks in mink farms [26]. Whether the mink virus can infect humans and cause efficient human-to-human infections is unknown.

Figure 2. Influenza virus evolution. Almost all IAV subtypes (H1 to H16, N1 to N9) have natural hosts in water birds, of which some have established infections in other species, such as H1N1, H2N2, and H3N2 in humans and pigs. Human influenza viruses have changed over the years mainly due to the emergence of four pandemic flu viruses. Currently, circulating influenza viruses include two IAV subtypes H1N1 and H3N2, and two IBV lineages (B/Yamagata and B/Victoria). Some animal IAVs, particularly bird and swine flu, can occasionally spill over to cause zoonotic infections in humans. In recent years, avian H5 and H7 viruses caused human infections with a high case fatality rate.

3.2. Mechanisms of influenza virus evolution – antigenic drift and shift

The evolution of influenza viruses is driven by two major mechanisms: antigenic drift and antigenic shift. Antigenic drift is the accumulation of mutations in the viral genome during viral replication owing to the lack of proofreading activity of viral RNA-dependent RNA polymerase [5]. Mutations located within the antibody epitopes of the surface envelope proteins, HA and NA, can reduce the recognition of pre-existing antibodies elicited against previous viral strains. Antigenic drift explains the occurrence of seasonal influenza infections and the need for annual influenza booster vaccines.

Antigenic shift is the result of the reassortment of viral genomic RNA segments of two different influenza viruses co-infecting the same cell to generate new strains and/or subtypes, which have the potential to cause severe disease and/or spread quickly in a population with no pre-existing immunity. The mechanism of influenza virus reassortment has been reviewed [27]. Antigenic shift or reassortment is a major driver of the generation of pandemic or zoonotic strains. The 1957 pandemic influenza virus resulted from the reassortment of an H2N2 avian virus and a human H1N1 virus, while the 1968 pandemic virus was the result of reassortment of an H3 avian virus and a human H2N2 virus then. The 2009 H1N1 pandemic virus (2009 H1N1pdm) was generated after multiple reassortment events in swine. Avian H5N1 and H7N9 viruses, which cause zoonotic infections in humans with high mortality, are generated through reassortment with other subtypes of avian viruses, especially avian H9N2 viruses.

4. Viral determinants for pathogenicity and virulence

Influenza replication capacity, disease severity, and transmissibility are both virus- and host-specific. Low-pathogenic avian influenza viruses cause mild symptoms in wild birds and poultry, with little or no signs of illness, whereas highly pathogenic avian influenza viruses cause high mortality in infected poultry. Compared to seasonal influenza viruses, pandemic strains cause high morbidity, mortality, and rapid transmission in the human population. Even among the pandemic strains, the 1918 virus is unique in its extreme virulence (estimated 1% mortality) and its distinct "W-shaped" curve of age-specific death rates, with peak deaths observed in young adults in addition to the elderly and infants [2]. Zoonotic infections by HPAIV H5N1 and H7N9 viruses led to 30% to 50% mortality in infected patients, but limited human-to-human transmission [3].

Extensive studies have been conducted to identify and characterize viral determinants associated with increased pathogenicity, virulence, and transmission. In particular, the genetic changes required for adaptive replication and airborne transmissibility in mammalian hosts [28–32] have been studied extensively (reviewed in [33]). The major virulence determinants (Table 1) include changes that affect viral entry, increase viral polymerase activity, and modulation of host responses. They function synergistically to cause efficient infection and sustained transmission in new hosts [46,68,95]. For example, a set of five or six substitutions in three viral genes (HA, PB2, and PB1) are required for an avian H5N1 virus to acquire airborne transmissibility [28,32]. Identifying viral outbreaks of pandemic potential through active surveillance of the prevalence and evolution of influenza A viruses in avian, swine, and human hosts to identify molecular markers of virulence is an important component of the pandemic preparedness plan.

4.1. HA variants expand tissue tropism and increase host cell susceptibility

HA is a major determinant of host range because of its essential role in host receptor binding and membrane fusion during viral entry. The HA trimer on the viral membrane consists of two domains: a globular head containing a receptor-binding site, and a membrane-proximal stem domain. The receptor-binding site consists of three secondary elements, the 130-loop, 190-helix, and 220-loop, and includes four highly conserved residues (Y98, W153, H183, and Y195) [96]. All the HA positions were based on H3 numbering [97]. HA mutants allow viruses to expand tissue tropism, switch receptor specificity, increase host receptor binding, and optimize membrane fusion at different temperatures or pH environments.

4.1.1. HA cleavage by host activating proteases
Low-pathogenic avian influenza virus (LPAIV) of H5 and H7 subtypes can mutate to HPAIV, mainly due to the acquisition of a multi-basic cleavage site [60]. Host protease-mediated proteolytic cleavage of the HA precursor molecules HA0 to HA1 and HA2 activates a conformational change in HA, which is essential for membrane fusion and viral infectivity [98]. Therefore, the distribution of activating proteases in the host is a major determinant of viral tissue tropism. The HA proteins of LPAIVs and mammalian influenza viruses contain a single basic residue at the cleavage site and

Table 1. Molecular markers associated with virulence and pathogenicity of influenza virus. HA substations are based on the H3 numbering system[97].

Genes	Subtype	Position	Molecular markers and functions	References
HA	H5	83, 128, 197, 496	K83, P128, K197, K496 work in two or more combinations to increase binding to α2–6 glycans	[34]
	H7	104	N104K increases affinity to both α2,3- and α2,6-linked SA	[35]
	H5	110	H110Y increases thermostability and pH stability, adaptive mutation for airborne transmission	[32,36,37]
	H5	126	S121N enables H5N1 virus binding to both a2–3 and a2–6 glycans	[38]
	H5, H7	138	L129V/A138V increase H5N1 binding to α2–6 glycans, A138V decreases α2–3 binding; A138-V186-P221 increased H7N9 binding to human-type receptors	[39–42]
	H5	143	G143R increases H5N1 virus binding to α2–6 glycans	[34]
	H5	158	N158D leads to loss of N-glycosylation site, increases human-type receptor binding, adaptive mutation for airborne transmission	[31,36,38,43]
	H5	160	T160A increases overall virus binding to both α2,3-SA and α2,6-SA, adaptive mutation for airborne transmission	[28,32]
	H7	186	A138-V186-P221 increase H7N9 binding to human-type receptors, V186G/K-K193T-G228S or V186N-N224K-G228S can switch the receptor specificity of the H7N9 HA from avian- to human-type	[39,44,45]
	H9	187, 227	T187P-M227L increase H9N2 binding to human-type receptors, enhanced replication and virulence in mice	[46]
	H1	190	D190-D225 determines α − 2,6 binding specificity of H1N1. E190-G225 switched to α − 2,3-binding	[47–49]
	H7	193	V186G/K-K193T-G228S can switch the receptor specificity of the H7N9 HA from avian- to human-type	[44,45]
	H5	196	Q196R/H increases H5N1 virus binding to α2–6 glycans	[29,34,50]
	H5	214	V214I increases H5N1 virus binding to α2–6 glycans	[50]
	H7	221	A138-V186-P221 increase H7N9 binding to human-type receptors	[39]
	H5, H7	224	V186N-N224K-G228S can switch the receptor specificity of the H7N9 HA from avian- to human-type, N224K increased H5N1 binding to human-type receptors, adaptive mutation for airborne transmission of H5N1	[31,35,44]
	H1	225	D190-D225 determine α − 2,6 binding specificity of H1N1. E190-G225 switched to α − 2,3-binding	[47,48,51]
	H2, H3, H5, H7, H9, H10, H15	226	Q226L-G228S change H2 and H3 receptor binding specificity from α2–3 to α2–6 glycans; Q226L increases HA binding to human-type receptors of various subtypes	[52,53,5439,55–58]
	H5, H9	227	S227N increases H5N1 virus binding to α − 2,6 glycans, T187P-M227L increase H9N2 binding to human-type receptors	[29,38]
	H2, H3, H5, H7, H9, H10, H15	228	Q226L-G228S change H2 and H3 receptor binding specificity from α2–3 to α2–6 glycans; G228S increased virus binding to α − 2,6 glycans	[39,52,53,55–58]
	H5	318	T318I increases HA stability, adaptive mutation for airborne transmission	[28,32,36,37,59]
	H5, H7	329	Insertion of multi-basic residues expands tissue tropism in birds and generates HPAIVs	[60,61]
	H5, H7	58 (HA2)	K58I increases thermostability and pH stability	[35,62,63]
PB2	H5N1	9	D9N enhances viral polymerase activity, inhibits MAVS to suppress IFN-I production	[64,65]
PB2	H1N1	271	T271A enhances polymerase activity in human cells	[66]
PB2	H7N9, H5N1, H3N2	526	K526R cooperates with E627K to synergistically increase viral polymerase activity	[67]
PB2		627	E627K is a mammalian-adaptive mutation, increases avian virus replication in mammalian cells	[68,69]
PB2	H9N2	526, 543, 627, 655	E627V-E543D-A655V-K526R mutations acted cooperatively to increase viral polymerase activity in human cells	[70]
PB2		701	D701N is a mammalian-adaptive mutation, increases avian virus replication in mammalian cells, improved binding of PB2 to importin protein in mammalian cells	[69,71]
PB2	H7N7	714	S714R is a mouse-adapted mutation, enhances viral polymerase activity in mammalian cells	[72]
PB1	H5N1	3	D3V enhances viral polymerase activity	[73]
PB1	H5N1	99	H99Y increases viral polymerase activity, mammalian-adaptive mutation	[28,32]
PB1	H1N1, H5N1, H7N9	612	K612 SUMOylation is critical for the viral RNA binding activity of PB1, essential for viral pathogenesis and transmission	[74]
PB1	H5N1	622	D622G enhances viral polymerase activity, virulence in mice	[73,75]
PA	H7N9	37	S37A increases polymerase activity in human cells	[76]
PA	H7N7	63	V63I increases viral polymerase activity, viral replication and virulence in mammalian cells	[77]
PA	H1N1	85, 186, 336	85I − 186S −336 M enhances avian virus polymerase activity in mammalian cells	[78]
PA	H5N1	224, 383	P224-D383 synergistically enhance viral replication in mammalian cells	[79]
PA	H7N9	383	N383D increases polymerase activity in human cells	[76]
PA	H5N6	343	A343T cooperates with E627K to synergistically increase viral polymerase activity in mammalian cells	[40]
PA	H9N2	356	K356R cooperates with E627K to synergistically increase viral polymerase activity	[67,80]
PA	H1N1	552	T552S increases avian virus polymerase activity in mammalian cells	[81]
NP	H7N9, H9N2	41	V41 enhances viral polymerase activity	[82]
NP	H7N9, H9N2	210	E210D enhances viral polymerase activity	[82]

(Continued)

Table 1. (Continued).

Genes	Subtype	Position	Molecular markers and functions	References
NP	H7N7	319	N319K is mouse-adapted mutation, improves binding of NP to importin protein in mammalian cells	[72,83]
NP	H1N1	357	Q357K enhances viral replication, mammalian-adaptive mutation	[84]
M1	H5N1	30, 215	N30D-T215A increases viral replication and viral virulence in mice	[114]
M1	H9N1	37	T37A abolishes the phosphorylation site to stabilize the M1 protein, increases viral replication in mammalian species	[116]
NS1	H5N1	42	P42S increases viral virulence in mice by preventing the dsRNA-mediated activation of the NF-κB and IRF − 3 pathways	[119]
NS1	H7N9	106	I106M increases viral virulence in mice by enhanced CPSF30 binding	[120,121]
PA-X			host- and strain-specific role in viral virulence and pathogenicity	[118]
PB1-F2			host- and strain-specific role in viral virulence and pathogenicity	[85,131]
PB1-F2	H5N1	66	N66S increases viral pathogenicity in mice	[94]

can only be cleaved by extracellular host proteases, which restricts viral spread to tissues where specific proteases are present. HPAIV HA proteins acquire a multi-basic cleavage site, which allows intracellular cleavage by ubiquitously present proteases, resulting in the systemic dissemination of HPAIVs and lethal disease in poultry.

4.1.2. HA-dependent host adaptation
Except for bat influenza viruses (HA17 and HA18), HA proteins (HA1–16) recognize terminal sialic acids (SA) of cell surface glycoconjugates as receptors. Avian and equine influenza viruses preferentially bind SA linked to galactose via an α − 2,3 linkage (α2,3-SA), whereas human influenza viruses prefer α − 2,6-linked SA and swine influenza viruses bind to both efficiently [99]. Differential receptor-binding affinity is an important determinant of the influenza virus host range and viral pathogenicity. α2,3-SA is prevalent in the intestinal epithelial cells of birds, allowing the efficient replication and shedding of avian influenza viruses. In humans, α2,6-SA is more abundant in the upper respiratory tract (URT) than in the lower respiratory tract (LRT), whereas α2,3-SA is more abundant in the lungs. Human influenza viruses primarily replicate in URT, causing mild disease, but efficient transmission among humans. Avian influenza virus infection in humans (zoonotic) replicates better in LRT than in URT, resulting in a more severe disease but limited ability for transmission [99]. Therefore, avian influenza viruses must acquire human receptor-binding specificity for efficient infection and airborne transmission in humans. Pigs have both α2,3-SA and α2,6-SA in the respiratory tract and can act as mixing vessels for avian and human influenza viruses [100].

Amino acids that determine receptor-binding specificity have been defined in many HA subtypes, which generally require two residue changes within the receptor-binding pocket, such as positions 190 and 225 for H1N1, 226 and 228 for H2N2 and H3N2 viruses [96].

For H1N1 viruses, D190 and D225 are associated with α − 2,6 binding, whereas E190 and G225 are associated with α − 2,3 binding [47,48]. The 1918 H1N1 pandemic HA protein, containing D190 and D225 residues, binds α − 2,6 receptors exclusively. D225G substitution led to mixed α − 2,6 and α − 2,3 binding, whereas D190E substitution alone resulted in α − 2,3 binding. Consistent with the α − 2,3 receptor-binding specificity, the recombinant 1918 pandemic virus with D190E/D225G mutations lost respiratory transmission between ferrets [49]. The recombinant 2009 H1N1pdm virus with D225G had dual receptor-binding specificity for α − 2,6 and α − 2,3-SAs and displayed increased binding to human LRT, but still retained aerosol transmission in mammalian hosts [51], which helps explain the severe disease associated with D225G during the 2009 pandemic. For H2N2 and H3N2 viruses, L226 and S228 are associated with α − 2,6 binding, whereas Q226 and G228 are associated with α − 2,3 binding [52]. Q226L and G228S substitutions switched receptor binding from α − 2,3 to α − 2,6, which contributed to the emergence of the 1957 H2N2 and 1968 H3N2 pandemic infections.

Q226L and G228S have also been shown to increase the α − 2,6 binding specificity of other avian HA subtypes (H5, H7, H9, H10, and H15) . However, switching these HA proteins to human receptor preference generally requires additional mutations [33,57] (Table 1).

Apart from substitutions to switch receptor-binding specificity, other mutations that increase HA stability and/or overall receptor binding are required for host adaptation. Site-directed mutagenesis and experimental adaptation of an avian H5N1 virus have shown that airborne transmissibility in ferrets requires only three to four substitutions in the H5 protein, apart from substitutions in polymerase genes [28,32]. Among the H5 substitutions, Q226L or G228S increased mammalian receptor-binding specificity, H110Y altered the acid and temperature stability of HA, and T160A increased overall receptor binding [32,36,37]. Another

experimental adaptation study [31] showed that a laboratory-generated reassortant virus requires four substitutions (N158D, N224K, Q226L, and T318I) within the H5 protein to be transmitted among ferrets through aerosol or respiratory droplets. N158D, N224K, and Q226L allow the switch to human receptor binding [31,36], while T318I increases the heat stability and decreases the pH threshold of HA during membrane fusion [37,59]. As the human-receptor-binding mutations in H5 reduce the HA heat stability and increase the pH threshold for membrane fusion, compensatory mutations such as T318I that reverse the effects can facilitate avian virus respiratory transmission in humans [101].

The emergence of H7N9 avian influenza viruses in China causing zoonotic infections with high CFR since 2013 has attracted considerable attention because of their continuing evolution and potential pandemic risk. Most zoonotic H7N9 isolates bind to both the human and avian receptors [102,103]. Q226L and G186V substitutions accounted for the increased α – 2,6 SA binding [39,56] but were not sufficient for the switch in receptor specificity. A combination of three substitutions, V186G/K-K193T-G228S or V186N-N224K-G228S, is sufficient to switch the receptor specificity of H7N9 from avian to human type [44], of which, G228S is a known determinant for human receptor binding, V186G/K/N loses the α – 2,3 binding and increases α – 2,6 binding, and N224K increases overall receptor binding [31,35,44].

4.2. Substitutions in the internal genes increase viral replication in target cells

Influenza virus internal genes, including three polymerase proteins (PB2, PB1, and PA), NP, and M1, contain important virulence markers that increase viral replication and progeny production. Polymerase genes play a critical role in the adaptive replication of avian influenza viruses [72]. These adaptive mutations in polymerase proteins generally increase the polymerase activity in mammalian cells. The major virulence determinant and mammalian adaptive marker is PB2 E627K [68,69]. Avian influenza viruses generally have E627, while all human viruses and many zoonotic influenza viruses have K627, except for the 2009 H1N1pdm virus. Another mammalian-adaptive substitution is PB2 D701N, which has been shown to expand the host range of the avian H5N1 virus to mice and humans and to increase viral transmission in guinea pigs [69,71]. Surprisingly, 2009 H1N1pdm had neither E627K nor D701N; the introduction of K627 and N701 did not enhance virulence or transmission in ferrets or mice [104].

Studies have shown that polymerase adaptive mutations, especially PB2 E627K, are caused by species-specific differences in ANP32 proteins, which are essential host factors for viral RNA synthesis [105–107]. Compared to avian ANP32A, human ANP32A lacks a 33-amino acid insertion in the C-terminal disordered domain, which allows the binding of PB2 K627, but not PB2 E627 [108,109]. Both human ANP32A and ANP32B homologs can support viral RNA synthesis by human influenza viral polymerases or avian viral polymerases with the PB2 E627K adaptive mutation [110,111]. Other polymerase mutations, such as PB2 K562R, PA A343T, and K356R, can cooperate with PB2 E627K to synergistically increase viral polymerase activity [40,67,80]. Another mechanism of adaptation has been shown for host importin-α isoforms, which mediate the nuclear import of vRNPs and viral proteins for viral RNA synthesis [112]. The avian PB2 and NP depend on importin-α3, whereas the mammalian PB2 and NP primarily use importin-α7 [112]. The PB2 D701N adaptive mutation enhances the interaction between PB2 and importin-α7 to promote vRNP nuclear import in mammalian cells [112,113]. Multiple virulence determinants or adaptive mutations have been identified in PB2, PB1, PA, and NP proteins, which function to increase avian viral polymerase activity in mammalian cells (Table 1), though their mechanisms of adaptation have not been well characterized.

As the major driver of viral budding and assembly, M1 also contributes to virulence by increasing viral replication. A previous study found that D30 and A215 contribute to the high pathogenicity of avian H5N1 viruses in mice and that the D30N/A215T double mutations significantly attenuated multiple H5N1 viruses in mice [114]. The M1 A215T substitution eliminates the SUMOylation of M1, leading to a significant reduction in M1 stability, vRNP nuclear export, viral replication, and viral progeny production [115]. The D30N substitution changes the shape of H5N1 viral particles from filamentous to spherical, although the mechanism by which the virion shape affects viral pathogenicity is unknown [115]. M1 T37A of the H9N2 virus abolishes the phosphorylation site to stabilize the M1 protein and increases viral replication in mice and human cells [116].

4.3. Viral antagonism of host antiviral immunity

Like all other viruses, influenza viruses must overcome host antiviral immunity for efficient replication. The viral NS1 protein is a key virulence factor known to impair host antiviral responses through various

mechanisms (reviewed in [117]). NS1 has been shown to interact with many viral and host factors through its functional domains, including an N-terminal RNA-binding domain (RBD) (aa 1–73), a linker region (aa 74–88), an effector domain (ED) (aa 89–202), and a C-terminal tail of variable length [118]. Many of the NS1 functions are strain-specific owing to the sequence variations of different NS1 proteins. A single substitution of P42S in the NS1 RBD prevented the dsRNA-mediated activation of the NF-κB and IRF – 3 pathways and drastically increased the pathogenicity of an H5N1 virus in mice [119]. The NS1 I106M substitution in an H7N9 virus increased its binding to CPSF30 and blocked host gene expression, leading to a more virulent infection in vivo [120]. Two NS1 mutations, D189N and V194I, present in circulating human H3N2 viruses impair their ability to inhibit host gene expression and attenuate viral virulence in mice, while NS1 V194I affects the thermosensitivity of viral replication and further attenuated viral virulence [120,121]. Recombinant influenza viruses expressing human NS1 (from human H1N1 or H3N2) induced higher levels of type I and III interferons (IFN-I/III) than those expressing avian NS1 (from avian H5N1, H7N9, and H7N2) in dendritic cells (DCs), suggesting that avian NS1 has an increased ability to antagonize IFN-I/III production [122].

PA-X protein is expressed from PA mRNAs via ribosomal frameshifting [123]. It has the same N-terminal 191 residues as PA, containing the endonuclease domain and a unique C-terminal domain, which is 61 aa long for most viral isolates, including the 1918 H1N1pdm and avian viruses, but is shorter (41 aa) in the 2009 H1N1pdm virus [123]. PA-X selectively targets cellular mRNAs, but not viral mRNAs for degradation, leading to host protein shutoff and inhibition of cellular antiviral responses [118,123]. PA-X also modulates host responses such as inflammation, immune responses, and apoptosis. The functional mechanism of PA-X in innate immune suppression is reviewed in detail [118]. Studies on PA-X-deficient influenza viruses have suggested that the biological roles of PA-X in viral replication and pathogenicity are host- and strain-specific [118]. PA-X was shown to decrease pathogenicity in mice by modulating host responses following infection with the 1918 H1N1pdm virus, the 2009 H1N1pdm strain (A/Beijing/16/2009 (BJ/09), HPAI H5N1 viruses, and circulating H1N1 strains [123–125]. Other studies, however, showed a pro-virulence role of PA-X in the 2009 H1N1pdm strain A/California/04/09, avian H9N2, and A/PR8 in mice [126–129].

PB1-F2 is a small protein expressed in the PB1 gene through the + 1 reading frame shift [130] and is a major virulence factor that modulates host innate immune responses (reviewed in [131]). PB1-F2 expression is detected in many, but not all, influenza A strains and is not detected in influenza B viruses [130]. PB1-F2 proteins vary in length and sequence. As such, the role of PB1-F2 in viral virulence is strain- and host-specific [131]. Different PB1-F2 proteins can differentially modify IFN-I responses, inflammatory responses, and immune cell death, contributing to viral virulence and pathogenicity [131]. A single mutation N66S in the PB1-F2 protein is associated with high pathogenicity of the 1918 H1N1pdm and HPAIV H5N1 viruses [94]. PB1-F2 of the 1918 H1N1pdm virus, but not of the A/PR8 virus, binds to DDX3 and causes its degradation [132], which may explain the severe pathogenicity of the 1918 pandemic virus. PB1-F2 of the highly pathogenic H7N9 virus, but not of the A/WSN virus, is a potent inhibitor of MAVS, an essential mediator of antiviral signalling, by forming aggregates in the mitochondria to prevent MAVS aggregation and activation, resulting in suppression of IFN-I production and MAVS-mediated NLRP3 activation [133]. H7N9 PB1-F2, however, does not affect extracellular NLRP3 inflammasome maturation, in sharp contrast to PB1-F2 of the A/WSN virus, which effectively suppresses IL – 1β processing and secretion by all stimuli [134]. Thus, the differential function of PB1-F2 may account for the highly elevated cytokine storm observed in the H7N9 infection but in not the WSN infection [134].

5. Host immune responses: immune protection or immunopathogenesis

The host immune response against influenza viral infections plays an important role in determining the disease pathogenesis [135]. A potent and fine-tuned immune response can effectively control and eliminate viral infection without significant damage, whereas an excessive and/or prolonged inflammatory response has been shown to cause tissue damage and disease exacerbation. Elucidating the mechanisms of immune protection and immunopathogenesis is crucial for the development of effective therapeutics against influenza.

5.1. Early activation of innate immunity correlates with protection

Upon infection, influenza viruses are recognized by the innate immune system through different classes of pattern recognition receptors (PRRs), membrane-bound

Toll-like receptors (TLR3, TLR7, and TLR8), cytosolic receptor RIG-I, and NOD-like receptor family NLRP3 [136]. Activation of the TLR and RIG-I signalling pathways leads to the upregulation of IFN-I/III and proinflammatory cytokines. IFNs act through paracrine and autocrine signalling to induce the expression of interferon-stimulating genes (ISGs), many of which exert antiviral activities to restrict viral replication. Proinflammatory cytokines activate phagocytic cells, recruit additional immune cells to the infection site, and contribute to the initiation of adaptive immunity. The NLRP3 inflammasome triggers the proteolytic cleavage and release of cytokines IL − 1β and IL − 18 and elicits pyroptosis in infected cells [136]. The protective roles of these innate immune pathways against influenza virus infection have been demonstrated in various knockout animal models. Some ISGs have demonstrated strong antiviral activity. Mx is a well-known restriction factor for the influenza virus. IFITMs block virus-host membrane fusion. The 2'−5'-oligoadenylate synthase (OAS) and RNase L degrade viral RNA. PKR binds to dsRNA and blocks protein translation by phosphorylating eukaryotic translation initiation factor 2α (EIF2α). Other antiviral factors have been identified that block influenza virus replication through various mechanisms (reviewed in [136]).

Early activation of robust innate immunity correlates with host protection against influenza viral infections in animal models. A highly pathogenic H7N9 virus caused complete lethality in mice with a high lung virus titer, whereas the H9N2 virus caused a mild and self-resolved infection. Compared to H7N9, H9N2 infection resulted in early and transient induction of innate immunity, as evidenced by the upregulation of IFN-Is, ISGs, immune cell markers, and proinflammatory genes [137,138]. Macrophages were recruited to the lungs at a much earlier time point (as early as 6 h) for H9N2 infection than for H7N9 infection (day 3) [137]. Co-infection with H9N2 resulted in effective protection against both H7N9 and PR8 (H1N1) infections, supporting the protective role of early innate immunity [137].

5.2. Pathogenic effect of accelerated activation of proinflammatory responses

Studies on severe influenza viral diseases in both humans and animals have demonstrated the pathological effect of hyperactivation of immune responses against elevated viral replication. The 1918 H1N1pdm virus is highly virulent and causes an abnormally high death rate in young adults, the elderly, and infants [2]. Mice and non-human primates infected with the reconstructed 1918 H1N1pdm virus have shown marked and prolonged activation of proinflammatory and cell death pathways, suggesting that accelerated activation of the host immune response contributes to severe pulmonary pathology [95,139]. Virological and immunological studies of human fatal cases of avian H5N1 and 2009 H1N1pdm infections suggest that a high viral load and the resulting intense inflammatory responses are central to the disease pathogenesis [18,140–142]. The avian H7N9 virus caused a more severe phenotype in animals than H9N2 and seasonal H3N2, and the disease severity correlated with increased infectivity and elevated induction of proinflammatory cytokines [143–146]. Excessive levels of proinflammatory cytokines, such as IL − 6, IL − 8, IP10, G-CSF, MCP − 1, and MIP − 1α, are associated with pulmonary inflammation and tissue damage in acute lung injury caused by influenza virus and SARS-CoV −2 infections, as well as in asthma and chronic obstructive pulmonary disease [95,145,147,148]. The immunopathogenic roles of cytokine storms and innate immune cells have been reviewed [149].

5.3. Antiviral and immunopathological roles of macrophages

Macrophages are important innate immune cells that protect against viral infections by secreting cytokines, initiating adaptive immunity, and clearing debris and dead cells [150]. Alveolar macrophages are tissue-resident macrophages that play an essential role in the protection from influenza virus-induced morbidity and mortality [86]. However, the excessive activation of cytokines in macrophages contributes to lung injury and disease severity [141,151]. Thus, the controlled activation of macrophages is critical for their protective role following influenza viral infections. Recent studies have shown that cellular Wnt/β-catenin/HIF −1α signalling and the transcriptional factor PPAR-γ function to promote the inflammatory activity of alveolar macrophages [90,152], and that downregulation of PPAR-γ following IFN-I antiviral signalling is critical for the suppression of exaggerated inflammatory response [152].

Macrophages are susceptible to influenza viral infection; however, most infections are abortive, and only a small number of viruses can produce infectious particles by overcoming cellular blocks [87,88,153]. Viruses that productively infect primary murine alveolar macrophages include a subset of highly pathogenic H5N1 viruses and A/WSN viruses. Even though productive replication in macrophages does not solely determine viral virulence in vivo, it decreases the

phagocytic function of macrophages and thus may contribute to disease development [88].

Influenza virus strains may also differentially modulate macrophage polarization, leading to either antiviral or pathogenic state [89]. Macrophages are highly heterogeneous and include two major subclasses: proinflammatory M1 and anti-inflammatory M2 macrophages. M1 macrophages are activated early in infection to secrete inflammatory cytokines, such as IL − 1, IL − 6, and TNF-α, and trigger antiviral responses. M2 macrophages are activated later during infection to terminate inflammation, repair tissue damage, and produce TGF-β and IL − 10 [154–156]. Recent studies have suggested that M2 macrophages are beneficial for influenza infection, while M1 polarization is associated with acute lung injury in severe influenza disease [155,157,158]. Thus, switching macrophage polarization from M1 to M2 May be a novel therapy for influenza diseases [159,160].

5.4. Antiviral and immunopathogenic roles of T cells

Both CD4 and CD8 T cells are important components of adaptive immunity to clear viral infections and protect the host from severe disease [161,162]. Pre-existing influenza-specific CD4 T cells are associated with lower viral shedding and less severe disease in healthy volunteers following influenza viral infection [91]. The adoptive transfer of memory CD4 T cells protects unprimed mice [92]. Memory CD8 T cells have also been shown to provide cross-reactive protection [93,163]. A comparison of host immunity in hospitalized patients after zoonotic H7N9 infection with diverse disease outcomes revealed an important protective role of memory CD8 T cells against severe influenza disease [164,165]. Patients with early recovery showed an early robust H7N9-specific CD8 T cell response, while those with prolonged hospitalization showed late recruitment of CD4 and CD8 T cells. In contrast, deceased patients showed minimal influenza-specific immunity and little T-cell activation [164]. These studies suggest that an early strong memory T cell response can help reduce disease severity. In contrast, overactive T cells can be harmful because they cause tissue damage and/or trigger inflammatory responses [162,166].

Tissue-resident memory T cells (T_{RM}) play an important role in the first-line defence against re-infection [167]. Lung CD8 T_{RM} cells mediate protection against respiratory infections by producing immediate effector responses at the site of pathogen entry; however, heightened responses may also cause inflammation and tissue damage [168]. Lung CD8 + T_{RM} cells have been shown to adopt exhausted-like phenotypes to avoid post-infection inflammation and fibrotic sequelae after influenza viral infection [169]. In aged hosts, however, lung CD8 T_{RM} cells exhibit malfunction in antiviral response and support chronic lung inflammation and fibrotic sequelae [170], which helps explain severe pneumonia in the elderly after influenza viral infection.

6. Host factors and cell signalling contribute to viral replication and pathogenesis

6.1. Pro-viral host factors

Like all other viral infections, influenza viruses rely on host factors and cellular machinery to reproduce (Figure 3). Hundreds of pro-viral host factors have been identified through genome-wide gene knockdown or knockout by CRISPR or RNAi, and through proteomic analysis of viral protein-associated cellular proteins [171]. Validating the functional roles of each candidate in influenza virus infection and characterizing their molecular mechanisms require tremendous effort and resources. Limited by space, only some proviral host factors are briefly summarized here.

Viral entry includes attachment, uptake, and fusion, and each step involves multiple host factors. Viral attachment requires sialic acids of cell surface glycoproteins or glycolipids. Viral uptake by endocytosis occurs through coordinated actions of cellular endocytic proteins. Host factors that promote influenza virus internalization include free fatty acid receptor 2 (FFAR2) and transmembrane protein immunoglobulin superfamily DCC subclass member 4 (IGDCC4) [172,173]. Once internalized, viral particles move from early to late endosomes, where fusion and uncoating occur to release the vRNPs. Prolidase (PEPD) is important for the early endosomal trafficking of viral particles [174]. The cysteine protease cathepsin W (CtsW) and lysosomal acid phosphatase 2 (ACP2) are required for viral fusion [175,176]. Cellular factor epidermal growth factor receptor pathway substrate 8 (EPS8) has been shown to promote influenza viral uncoating [177].

Influenza virus components must travel between the cytoplasm and the nucleus, as viral RNA synthesis occurs in the nucleus, while viral protein synthesis and viral particle assembly occur in the cytoplasm. The influenza virus utilizes the cellular nucleocytoplasmic trafficking machinery for the nuclear import and export of vRNPs, viral mRNAs, and viral proteins [178]. Nuclear importation depends on cellular importin proteins [179] and is enhanced by G protein
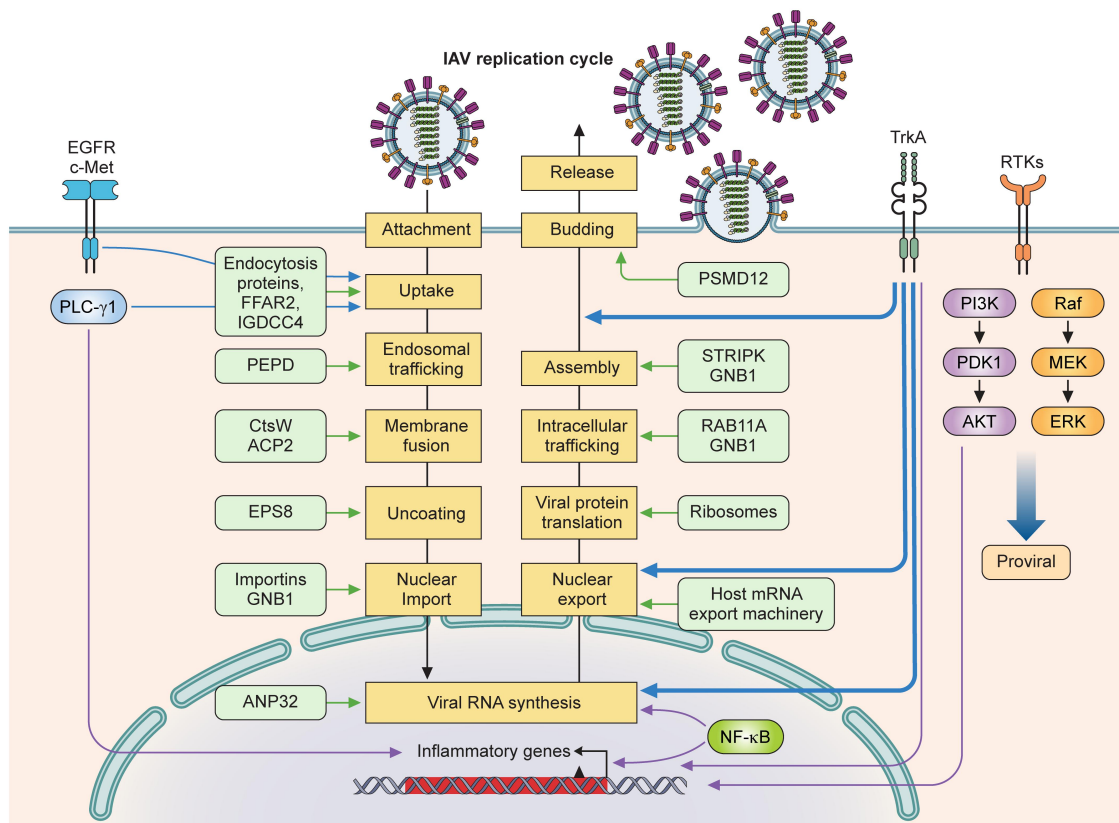
Figure 3. Pro-viral host factors and host signalling pathways important for influenza viral replication. Host factors and cellular signalling pathways are hijacked by the influenza virus to promote viral replication at different steps of the viral life cycle. Host factors are listed inside light green boxes next to the specific step of viral replication. Receptor tyrosine kinases (EGFR, c-Met, TrkA) on plasma membranes are activated by influenza viral infection and function to promote viral replication. EGFR, c-Met, and PLC-γ1 enhance viral uptake. TrkA signalling is important for several steps of the viral life cycle: viral RNA synthesis, vRNP nuclear export, and viral budding and release. NF-κB signalling enhances viral RNA synthesis and induces the expression of proinflammatory genes. PI3K/Akt and Raf/MEK/ERK pathways also strongly increase viral replication.

subunit β1 (GNB1), which increases the interaction between PB2 and importin proteins [180], and by BinCARD1, an isoform of Bcl10-interacting protein with CARD (BinCARD), which increased the binding of NP with importin α7 [181]. The nuclear export of viral mRNAs utilizes the host mRNA export machinery to translocate through the nuclear pore complex for translation in the cytoplasm [182]. Many cellular factors are involved in different steps of viral RNA synthesis and have been reviewed [183]. Newly synthesized vRNPs must accumulate at the plasma membrane for assembly. Cytoplasmic trafficking of progeny vRNPs depends on their association with the cellular Rab11 GTPase isoform RAB11A [184] in recycling endosomes along the microtubule network, alternative vesicles, or liquid-phase organelles (reviewed in [185]).

The viral matrix proteins M1 and M2 play a central role in viral assembly and budding. M1-interacting host factors have been shown to modulate their functions. GNB1 binds to M1 via intracellular trafficking to the

plasma membrane and promotes viral release [186]. PSMD12, a 26S proteasome regulatory subunit, mediates K63-linked ubiquitination of M1 at K102 to promote viral budding [187]. M1 phosphorylation at the T108 site increases multimerization at the cell membrane and controls its binding affinity to the cellular striatin-interacting phosphatase and kinase (STRIPAK) complex, which is important for M1 polymerization and viral replication [188].

6.2. Pro-viral host signalling pathways

In addition to antiviral responses, various cellular pathways are activated by influenza virus infections and exploited to support viral replication. Receptor-tyrosine kinases (RTKs) and their downstream signalling pathways (Raf/MEK/ERK, phosphatidylinositol – 3-kinase (PI3K)/Akt, JAK/STAT, PLC-γ1, NF-κB) have been found to facilitate viral replication through different mechanisms [189]. Influenza virus infection activates many RTKs, such as epidermal growth factor

receptor (EGFR), c-Met, and tropomyosin receptor kinase A (TrkA), possibly through clustering of lipid rafts induced by multivalent virus binding [190,191]. Activated EGFR and c-Met promote efficient viral uptake [190]. EGFR inhibition by small compounds or the protein antagonist SOCS5 reduces influenza virus replication [192,193]. In addition to directly promoting viral entry, EGFR activation has been shown to suppress IFN-I/III production and antiviral response [194,195], further increasing viral production. However, the role of EGFR signalling in influenza disease could be complex. EGFR has been predicted to be a key regulator of viral pathogenicity by network-based analyses of transcriptomic and proteomic data from mice infected with six influenza virus strains with differential disease severity [196]. Treatment of virus-infected mice with the EGFR inhibitor gefitinib caused more body weight loss after non-lethal infections, but not after lethal infections [196], suggesting an antiviral role of EGFR during non-lethal infections.

TrkA is another RTK activated by influenza virus infection but has post-entry functional roles in the viral replication cycle [191]. TrkA and its high-affinity ligand, nerve growth factor (NGF), are essential for neuron cell development and functions [197]. TrkA is also expressed in a wide variety of non-neuronal tissues and cell types, including human lung epithelial and endothelial cells [198], and plays an important role in allergic airway inflammation [199,200]. TrkA inhibitors block influenza virus replication at multiple steps after viral entry, viral RNA synthesis, vRNP nuclear export, and viral budding and release [201,202]. Using a TrkA-[F592A] knock-in mouse model to specifically control TrkA kinase activity [203], Verma et al. showed that TrkA not only promotes influenza virus replication in airway epithelial cells but also contributes to airway inflammation and lung pathology by activating the expression of proinflammatory cytokines/chemokines in infected cells [191]. Thus, targeting TrkA could be an effective treatment against influenza viral diseases by blocking both viral replication and airway inflammation.

NF-κB is a major mediator of proinflammatory cytokines, which can exert antiviral activities [204,205] or mediate pathogenic inflammatory responses [206,207]. Interestingly, many studies have demonstrated a pro-viral role for NF-κB in influenza virus replication [208–212], specifically in promoting viral RNA replication [208,209,213,214], suggesting that influenza viruses can convert this antiviral and proinflammatory pathway for viral replication. Similar findings were observed for PI3K, whose activation by influenza viral infection [215–217] was shown to promote IFN-I production through RIG-I

signalling [217]. PI3K inhibitors, however, have been found to strongly suppress influenza virus replication at multiple steps of the viral life cycle [218–220], demonstrating a pro-viral role of PI3K signalling. In addition, inhibitors of the Raf/MEK/ERK pathway have been shown to strongly inhibit IAVs and IBVs in vitro and in vivo [221–224]. Phospholipase C gamma 1 (PLC-γ1) signalling is virus- and cell-type-specific. Activated PLC-γ1 has an important pro-viral role in the cellular uptake of H1N1, but not H3N2 virus, in epithelial cells [225], while it exerts an antiviral and inflammatory role in H1N1-infected macrophages by activating NF-κB to express inflammatory cytokines in a positive feedback loop [226].

Viral infection is expected to activate the host signalling network, leading to various cellular responses, such as stress, inflammation, and cell death, which can restrict viral infection (antiviral) and cause disease symptoms (proinflammatory). Influenza viruses not only antagonize antiviral responses but also hijack the activated signalling network to efficiently accomplish different steps of the viral life cycle (pro-viral role). Thus, targeting the pro-vial and/or proinflammatory pathways in the influenza virus-infected cells can be developed as effective anti-influenza therapeutics [191,210–212,227], which, unlike inhibitors of single viral or host targets, generally has a high barrier to resistance [193,201,210]. The roles of host signalling pathways in viral replication and disease severity, however, can be virus- and cell-type-specific and need to be carefully evaluated for therapeutic development.

Summary

The continuing evolution of influenza viruses, driven by antigenic drift (mutation) and shift (reassortment), is a major barrier to the development of effective vaccines and antiviral therapeutics. Severe influenza is caused by direct viral cytopathic effects and immunopathogenesis. This review summarizes viral determinants and host components that affect influenza virus pathogenicity and virulence. Further studies are needed to characterize the molecular mechanisms of viral virulence determinants and pro-viral host factors, elucidate the complex roles of host immunity and cellular pathways in viral replication and disease development, and identify the control mechanisms of the protective and pathogenic roles of host signalling and immune responses. This knowledge will facilitate the development of new classes of antiviral drugs targeting different viral and host components as well as novel therapeutics that exploit antiviral roles and dampen the pathogenic effects of host signalling and immune responses.

Data sharing statement

Data sharing does not apply to this article, as no new data were created or analysed in this study.

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