Interactions between influenza A hemagglutinin and host cell receptors

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Annual outbreaks of influenza infection are an ongoing public health threat, which kill thousands of people worldwide. Influenza A virus is the most clinically important subtype of the influenza virus. It is the cause of the four pandemics that occurred in the 20th and the 21st century and still causes thousands of deaths worldwide annually. Understanding the life cycle of influenza virus is a prerequisite to competing it. Inhibiting the virus before infecting the host cell eliminates the need of drug delivery into cells and prevents the storm of cytokine expression accompanying the virus infection, which is believed to be the cause of severe consequences of influenza infection. Consequently, viral entry, the first step during infection, is our interest. This is a systematic review where 110 manuscripts were included. Google scholar was the search engine with keywords that included influenza A virus, influenza A virus receptors, influenza A, and host cells. The aim of this review is to give an overview of the multiplicity of factors and cofactors that orchestrate the process of viral entry and to mark its features.

Keywords:
glycosylated receptors, host cell receptors, influenza A virus

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Introduction

Influenza virus is one of the members of the orthomyxoviridae family which consists of influenza A, B, C, and other genera [1]. Subtype A is the most clinically important subtype. It is the cause of the four pandemics that occurred in the 20th and the 21st century and still causes around 500 000 deaths worldwide annually from seasonal flu [2–4]. It infects a vast variety of hosts such as human, swine, whales, birds, and other mammals. This wide range of hosts gives influenza A a chance to spread, mutate, and reassort causing continuous changing in antigenicity. Thus, it can easily escape the vaccine immunity and the natural immune system [5–8]. Understanding the life cycle of influenza virus is a prerequisite before competing it. Inhibiting the virus entry before infecting the host cell eliminates the need of drug delivery into cells and prevents the storm of cytokine expression accompanying the virus infection, which is believed to be the cause of severe consequences of influenza infection [9]. The aim of this review was to give an overview of the multiplicity of factors and cofactors that orchestrate the process of viral entry and mark its features.

Materials and methods

This is a systematic review. After approval of the ethical committee of the department. Articles were collected using the Google scholar database; a high number of articles were retrieved from this database compared with PubMed and Cochrane databases. Articles were collected based on the inclusion criteria using keywords, such as influenza A virus, influenza A virus receptors, influenza A virus, and host cells. Articles were collected from any journal, any time periods, and any study type have the keywords but should be full text only.

We collected 180 articles; 70 were excluded because the data was not relevant to the core of the review, for example, data including immunological responses to viral infections, steps of viral replication and transcription, viral exit, and host cell responses. The data was extracted using the same data extraction spreadsheet with a dropdown menu which had the following questions: what is the main factor in viral entry? What are the main host factors which help virus entry regarding the topology, glycosylation of the receptors? What are the other helping factors in influenza virus entry? What are the viral factors required for viral entry? Data had been extracted without adding additional information, so it could be readily compared. As an internal quality control,

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each paper was reviewed by two reviewers. Each reviewer completed data extraction separately, compared afterwards, and any discrepancies had been resolved by a third reviewer.

Hemagglutinin, the main factor in viral entry

The main factor in viral entry is hemagglutinin (HA). HA is a homotrimer glycoprotein on the viral envelope. It is first synthesized as a precursor, HA0, and then cleaved into HA1 and HA2, which remain linked by a disulphide bond [10,11]. Although 18 HA subtypes are currently well known, their corresponding receptors are still under research. Sixteen subtypes are found in waterfowls while HA17 and HA18 subtypes are found in bats [12,13].

There are different viral and host cellular factors involved in viral entry. Viral factors include the receptor-binding domain (RBD) and glycosylation sites around the receptor while host cellular factors include sialic acid receptors, different linkages and topologies, and desialylated receptors [14]. The interaction between HA which exist on the viral envelope and sialic acid on host cells is believed to be the first step of viral entry; therefore, HA is the main contributing factor in the entry step [15,16].

Viral factors involved in viral entry

Receptor-binding domain and preference

The RBD are highly conserved in all HA subtypes; they are located at the globular head at the distal end of HA1 and are surrounded by glycosylation sites. Two decades ago, it was believed that only eight conserved residues are present in HA RBD and are responsible for interactions with sialic acid and preference to humans or non-humans. Their names are based on H3 numbering which are 97, 98, 134, 139, 153, 183, 184, and 195 [17]. However, current studies prove that this process is more complex and additional residues are involved in binding.

Human and avian viruses differ in 226 and 228 residues that are present in the RBD, which correlate with their affinity for either α2,6 receptors in humans or α2,3 receptors in birds. Human viruses have a leucine and serine residue at positions 226 and 228. On the other hand, avian viruses have a glutamine and glycine at those positions [18–20]. However, in H1N1 residues 226 and 228 do not seem to have an effect. Substitutions from asparagine to glutamate at position 190 and asparagine to glutamine at position 225 are responsible for the altered binding preferences [21]. In subtype H3, residues 193 and 218 appear to have higher importance in preference when compared with residues 226 and 228 [22]. In another study, sequencing of human and avian H1 viruses showed that they differ in their amino acid residues at position 186 and 225. Proline at position 186 and glycine at 225 prefer α2,3 receptor binding while serine and asparagine at position 186 and 225 prefer binding to α2,6 receptor, respectively [23]. Moreover, residues 226 and 228 do not play a direct role in binding as they do not bind directly with the host cell receptor, but they affect the contour of the pocket [24,25]. Therefore, residues 226 and 228 have an indirect role in binding preference through altering the pocket shape to correlate with the topology of glycans. Alterations in the conserved residues (307, 310, 220, and 161) of subtype H5N1 (A/Vietnam/1203/04) affect the tropism and binding process by abolished binding, reduced binding, or changing receptor preference [26]. The conserved residues were substituted by alanines to test their binding ability to red blood cells (RBCs) of three different animal species. The RBCs of horses, swine, and chicken express different sialic acid receptors on their surface. Substitutions at position K307 and /K310 in combination abolish binding for all RBCs while R220A substitution only reduced the receptor binding. Interestingly, substitution of tyrosine with alanine at position 161 changes the affinity to *N*-glycolylneuraminic acid of RBCs from RBCs of horses and swine but not from chicken RBCs [27]. All these alterations and differences reflect the complexity of the RBD and the variable behavior among different subtypes of influenza A virus.

Glycosylation assisting in viral entry

Glycosylation are expressed around RBD and have numerous functions for viruses. For instance, glycosylation of the antigenic sites protect the virus by interfering with the antibodies [28,29]. They can also give rise to antigenic drift [30] or act as modulators for proteolytic processes at the cleavage site of the flu virus [31,32]. Moreover, glycosylation are required to maintain the stability of HA and the fusion of HA with the endosomal membrane [33]. Moreover, glycosylation also greatly affect viral entry by their significant variation in number and structure around the RBD of HA in different influenza subtypes [34]. This has been shown in high pathogenic avian influenza viruses after deletion of two N-linked oligosaccharides, named ASN123 and ASN149. The affinity of HA to the receptor strongly increased and could not be released by neuraminidase. On the other hand, sialylation of these oligosaccharides after expression and in the absence of neuraminidase abolishes adsorption [34]. Consequently, glycosylation are present around the receptor-binding

site to control the affinity of binding. Glycosylation sites are frequently mutated; therefore, the flu virus is adapted to transmit from host to host which leads to subsequent changes in receptor preference [35]. For example, H1N1 USSR/90/77 virus, changed Asn131 to Asp131 in the glycosylation site, and subsequently lead to the abolished preference to α2, 6 receptor [36].

The abundance of these glycosylation sites may be another factor for viral entry as high glycosylated viruses show better entry than their low glycosylated peers. In particular, this was obvious in cells expressing entry receptors other than sialic acid [37]. The type of oligosaccharides might also play a role as viruses of high mannose oligosaccharides show increased entry into macrophage cells that express the macrophage mannose receptor (MMR) [38,39].

Other proteins on the virion surface

Furthermore, neuraminidases NA and M2 are other proteins on the virion surface that have active roles in the process of entry and cooperate with HA to help in the entry. Neuraminidase surface proteins on the viral envelope are known for their importance in influenza virus egress [40] and also have a role in viral entry. In fact, they are necessary for the binding activity of HA [23] to its receptor. HA of the highly pathogenic avian influenza was unable to be adsorbed to the cell surface but treatment of the cell line with bacterial NA results in extensive hemadsorption. H1N1 A/WSN/1933viral strain with an oligosaccharide of a complex type beside the RBD showed enhancement of hemadsorption of HA after treatment by NA [34]. In contrast, HA RBD hemadsorption of the Hong Kong type was not affected by NA treatment. This virus has an oligosaccharide of high mannose type besides the RBD. The affinity of fowl plague virus HA to the receptor is negatively affected by the adjacent oligosaccharides; this interaction allows binding and elution from the receptor to take place. Furthermore, neuraminidases may have an enhancement role by acting on the plasminogen and sequestering it to the plasmin which then cleaves HA and leads to active infection [41,42]. However, another study using the 1918 NA virus did not support this role and suggested that this function might be virus specific [42,43].

M2 is the third surface protein on the viral envelope which helps in uncoating in order to complete the entry process. Proton channel protein (M2) is presented as a homotetramer and serves as a proton-selective ion channel protein. It becomes active at low pH and allows an influx of H^+ ions [44] into the virion. This disrupts the attachment between the matrix protein (M1) and the ribonucleoprotein complex. This disruption facilitates the uncoating process and releases ribonucleoprotein into the cytoplasm which marks the completion of the entry process.

Host factors involved in viral entry

Host cell receptors

The interaction between viral envelope HA and receptors on the host cell surface is the initiation step for viral infection [45]. Hence, the surface of host cell receptors has a great role in viral entry. Around 60 years ago, Gottschalk and colleagues established a link between influenza entry and sialic acid [46]. Nowadays, it is well known that the minimum binding requirement for viral entry is the presence of terminal sialic acid on the host cell [47–49]. Sialic acid is a nine carbon monosaccharide family linked to the surface of host cells by glycolipids or glycoproteins [16]. Over the last few decades, extensive research has been done to explore the fine structure and interaction between HA and sialic acid. More than 20 different types of sialic acid have been found, making the structure of sialic acid in glycolipids and glycoproteins highly diverse [50], and consequently affects viral preference. *N*-acetyl-nuraminic acid and *N*-glycolylneuraminic acid are the most common sialic acids in mammals and both are influenza ligands. *N*-glycolylneuraminic acid is not synthesized in humans due to the lack of the responsible gene; however, the gene is present in other mammals, such as apes [51]. The three most prevalent sialic acid types, called *N*-acetylneuraminic acid, *N*- glycolylneuraminic N- acid and 9-*O*-acetylneuraminic acid, were tested on 18 different influenza A different species of humans, swine, equine and avian viruses [52]. It has shown that all 18 viruses are able to agglutinate RBCs carrying *N*-acetyl neuraminic acid α2,3 (NeuAc α2,3) or *N*-acetyl neuraminic acid α2,6 (NeuAc α2,6). Changing the receptor to *N*-glycolylneuraminic acid makes a difference in binding, some have no effect on the binding of human, swine, equine, or avian viruses while others lose binding [52]. Alteration of the receptor site to 9-*O*-acetyl-*N*-acetyl neuraminic acid results in loss of agglutination for all viruses except one avian H3 type virus named A/duck/Mallard/NY/6874/78 [52]. The presentation of sialic acid in either glycoprotein or glycolipid may somehow affects viral entry. Influenza virus shows preference to the expression of sialic acid in glycoprotein but still has the ability to bind to both [23]. Glycolipid is not essential for infection *in vivo* and the level of flu infection is not affected in cell lines deficient in glycolipid, such as the GM 95 cell line [53].

N-linked glycosylation

N-linked glycans is another significant factor that affects viral entry. Infections by the viral subtypes A/WSN/33(H1N1), A/Udorn/307/72(H3N2), and B/Yamagata/78 in mutant Chinese hamster ovary cells with absent glycans (Lec1 cells), lead to infection abortion [54]. The absence of infection in these cells, which are deficient in *GnT1* gene required for *N*-linked glycans, highlights the importance of *N*-linked glycans because the virus can attach to the cells in the presence of sialic acid but does not become endocytosed or internalized from the plasma membrane [55]. Infection and replication of the virus is restored in Lec1 cells after the expression of wild type of GnT1 [55].

Sialic acid linkage

Sialic acid linkage is another important factor altering viral entry into host cells. Twenty years ago, the basic rules for influenza virus binding has been set clearly: human viruses recognize the SAα2,6 receptor; avian viruses recognize the SAα2,3 receptor, and swine viruses recognize both [17,48,56,57]. These rules correlate well with the distribution of sialic acid linkages in different hosts. The epithelium of the human airway expresses Neu5Ac α2,6 Gal, while the epithelium of duck intestine expresses Neu5Ac α2,3, and the epithelium of swine trachea expresses both [57,58]. In 1998, H5N1 was first appeared to be able to replicate in humans [59,60] in spite of lacking affinity to human receptors [24]. Then, it became clear that the human respiratory epithelium expresses both receptors and has a sufficient level of α2,3 expression for making productive avian infection [61,62]. This explains the outbreak of avian flu H5N1(A/HK/156/97) in humans in Hong Kong in 1997; the virus did not change preference and remained bind to the α2,3 receptors. The preference of H5N1 to α2,3 receptor persisted until the isolation of variants in 2003 (A/Vietnam/30262III04 and A/Vietnam/3028II04). It was discovered that HA has mutations at position 182 and 192; thus, it has the ability to bind to both α2,3 and α2,6 receptors [24,60]. However, the specificity of the receptor is not absolute, and it could be overcome by using a high viral load. Horimoto and Kawaoka [63] explained the 'specificity leakiness' and stated that the specificity of the receptor is only preferential and not absolute [58]. This clarified the ability of some avian viruses to act as human flu viruses in receptor binding. For instance, H9N2 poultry lineage virus has the ability to bind to α2,6 receptors [64]. The reverse could also happen as the replication of human α2,6 virus in the respiratory tract of mice is possible after knocking down sialyltransferase and in the absence of α2,6 receptors. This elevates the attention toward the presence of other moieties or the ability to bind to α2,3 by low affinity [65].

Interestingly, the quail and chicken intestinal epithelial cells contain both types of receptors, α2,3 and α2,6, whereas duck epithelial cells express only the α2,3 receptors [66–68]. Extensive studies on the respiratory tract, the primary target for flu viruses, were done in order to elucidate the distribution of the receptors which help in viral infection [69]. These studies were done using either human or ferret respiratory tissue [70] which are highly similar in their receptors. Flow cytometry studies were done using lectins for the identification of α2,6 and α2,3 receptors, and fluoresceinated viruses H3 for identification in the human bronchial tree [58]. The studies showed that the two linkages are present in the human respiratory system with more abundance of α2,6 in the upper respiratory epithelium [57,58,71,72]. They also showed the presence of α2,6 receptor in ciliated and nonciliated cells, as well as the occurrence of α2,3 in ciliated, goblet and basal cells, and one-third of ciliated cells express both receptors [70]. Furthermore, they demonstrated the presence of α2,3 in bronchial mucin, which inhibits the binding of α2,3 viruses more than α2,6 viruses [16]. Another study confirmed the detection of α2,6 *O*-linked glycans also in goblet cells at the apex of the tracheal epithelium [73].

The inner structure of receptors

Inner structures include three factors: bond types between the galactose and the following sugar residue (β1-3 or β1-4), the residue nature of *N*-acetyl galactosamine(GalNAcα) or *N*-acetyl glucosamine (GlcNAcβ) and changing the constituents at the ring of GlcNAc [74]. These differences and their effect on binding affinity are detected by testing different viruses using competitive solid phase-binding assays [64]. Wild duck viruses show high affinity to β (1-3)linkage, equal affinity for nonsulfate and sulfate receptors, and no affinity to fucosylation. It can also bind to GlcNAc or GalNAc [75]. American avian (H7N2) and Eurasian human (H7N7) show enhanced affinity to sulfate receptors, 1–4 linkage, and GlcNAcβ core. Human isolate viruses A/Hong Kong/1073/99(H9N2), A/USSR/039/68, and A/Canada/228/68 bind strongly to Neu5cα2-6Gal but not to α2,3 linkages [64]. Four types of swine viruses were tested and show affinity to1–4 linkage, fucosylated and sulfated GlcNAcβ, α2,3 receptors and moderate affinity to α2,6 receptors [76]. Interestingly, the ability of many poultry viruses to bind to the common receptor [6 sulfo sialyl lewis x

(Neu5Acα2,3 Galβ1-4)] is accompanied also by having the affinity to bind to the human-type (NeuA5cα2-6Gal) receptor. This dual affinity has been shown for H1, H3, H9 avian-like viruses, H9N2 Eurasian poultry virus, and North American and Eurasian H7 viruses. All of these enhance the potential threat of transmission of avian viruses to humans [64]. H5N1 subtypes show high affinity to α2,3 sulfate receptors with some of them showing increasing affinity by an added fucose in the 1–4 linkage GlcNAcβ [64].

Sialic acid topology

The topology of sialic acid is another important factor in receptor specificity, which was discovered by analysis of the co-crystal structures of HA-glycan. The topology and conformation of receptors play an important role in the specificity of human and avian viruses [73,77]. Molecular dynamic simulations, using different H3, H5, and H9 viruses, proved topological differences between avian and human receptors on binding to HA. This topology determined the specificity of HA and showed that the dynamic properties of the receptor is strictly related to its structure [78].

Avian viruses prefer sialic acid in the shape of cone; it exists in both α2,3 and α2,6 receptors with short glycans. Human viruses, however, prefer umbrella-shaped SA which only occurs in α2,6 receptors with long glycans [73]. This explains the limitation of H5N1 virus in human-to-human transmission as H5N1 can only bind to α2,3 or α2,6 receptor with short glycans, but not to α2,6 receptors with long glycans. On the other hand, Nicholls *et al.* [79] demonstrated that H5N1 can infect the upper and lower respiratory tract with or without α2,3 receptors. In addition, binding of H5N1 to α2,6 receptors with long glycans was detected with very high viral concentration. Nevertheless, the affinity is minimal compared to α2,3 receptor in the concentration range of the entire virus [79].

In α2,3 receptors, the Neu5Ac at the non-reducing end of the receptor is responsible for the major contact with avian HA [73]. After binding to the HA-binding pocket, the remaining residues in the trisaccharide start to make torsions at their glycosidic junctions, so that the glycan forms a cone shape. In contrast, the longer glycans in human receptors give more torsion and free movement that lead to the configuration of umbrella topology. The receptors form these configurations only after binding to the HA, while in their free states their shapes depend on the connectivity of their non-reducing ends [73,80].

DC-sign and L-sign receptors

The interactions between the flu virus and the receptor as well as viral attachment are proved to be a multistep process that requires multiple factors [23]. Other receptors that mediate entry are still unknown and/or under research. On the contrary, cell receptors that depend on calcium to help viral entry have been recognized [81,82]. DC-sign and L-sign are type II transmembrane proteins that contain a domain rich in C-type carbohydrate, and can interact with viral receptors that are rich in oligosaccharides [38,39]. The expression of these proteins on a mutant cell line Lec2 Chinese hamster ovary cells, which is deficient in sialic acid and resistant to flu virus infection, rendered the cell permissible for infection by H3N2(BJx109). In contrast, PR8 (H1N1) with low levels of mannose glycans showed low efficiency in infecting these cells [37]. Human cells that express C-type lectins can support infection efficiently. Without the help of SA, DC-sign and L-sign receptors act as viral entry receptors of many other viruses, such as Ebola [83], dengue [84], SARS Cov [85,86], HIV-1 [87,88], West Nile virus [89], and hepatitis C virus [90]. Subsets of the human lung stem cells, lung endothelial cells, alveolar cells type II and epithelial cells of the bronchiole express L-sign [91,92] and can be infected by viruses as well [93,94]. Thus, the L-sign is most probably recognized by the virus and leads to success of entry and virus spread. On the other hand, the DC-sign is expressed by populations of dendritic cells in the lung and macrophages of the alveoli [95–97], and recognizes mannose-rich glycans. Moreover, the DC-sign has only an affinity to oligosaccharides with fucosylation [98]; therefore, differences in glycosylation around the viral RBD may have a role in the recognition and internalization by these receptors.Several studies proposed that the presence of abundant sialic acid on the cell surface and the attachment of the virus to it is not necessarily followed by viral entry and infection [99]. Despite the role of sialic acid in flu virus binding, the entry of flu virus into asialated cells has been reported [14].

Macrophages as viral targets

Macrophages, as part of the innate immune system, detect the flu virus in early stages of infection [100,101]. Reports indicate the capability of the human macrophage to prevent productive viral infection [102–104]. Macrophages do not support viral replication or release of progeny [105]. Macrophages also release inflammatory cytokines and interferons against flu virus are considered the end stage for viral infection. The MMR, a mannose

C-type lectin, was recognized as a calcium-dependent receptor on the surface of macrophages which allows viral entry and infection but not causing the disease [106,107]. Infection by MMR involves virus attachment to sialic acid on the MMR followed by recognition of viral oligosaccharide by the lectin activity of MMR [108]. Another C-type lectin receptor on macrophages is the macrophage galactose-type lectin [108]. This integral membrane glycoprotein allows virus internalization into macrophages [109,110]. Poorly glycosylated virus A/PR/8/34 shows a lower level of infection than the highly glycosylated siblings, even though the virus attaches equally to the surface of the cell by SA. This confirms that high mannose C-type lectins play a significant role during macrophage infection [107].

Final remarks

All the previous interactions between the virus and cellular receptors give us an idea about the complicated process of viral attachment and subsequent entry and the multiplicity of factors affecting it. The ability of these viruses to reach their targets and infect the cells are due to the genetic drift and shift. The envelope of the influenza virus is engendered from the infected cellular membranes during virus egress which may explain its ability to use many cellular factors for interaction with cells. The complexity of these interactions raises the need for continuous research to deepen our understanding of viral entry and hopefully will help in finding newly therapies against viral infections.

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Conflicts of interest

There are no conflicts of interest.

References

1 Hause BM, Collina EA, Liu R, Huang B, Shenge Z, Lu W, *et al.* Characterization of a novel influenza virus in cattle and Swine: proposal for a new genus in the Orthomyxoviridae family. MBio 2014; 5:e00031-14.

2 Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918-1920 'Spanish' influenza pandemic. Bull Hist Med 2002; 76:105–115.

3 Shao W, Li X, Goraya MU, Wang S, Chen JL. Evolution of influenza a virus by mutation and re-assortment. Int J Mol Sci 2017; 18:1650.

4 Rumschlag-Booms E, Rong L. Influenza a virus entry: implications in virulence and future therapeutics. Adv Virol 2013; 2013:9.

5 Guo YJ, Jin FG, Wang P, Wang M, Zhu GM. Isolation of influenza C virus from pigs and experimental infection of pigs with influenza C virus. J Gen Virol 1983. 64:177–182.

6 Osterhaus ADME, Rimmelzwaan GF, Martina B, Bestebroer TM, Fouchier RAM. Influenza B virus in seals. Science 2000; 288:1051–1053.

7 Scorza FB, Tsvetnitsky V, Donnelly JJ. Universal influenza vaccines: shifting to better vaccines. Vaccine. 2016; 34:2926–2933.

8 Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev 1992; 56:152–179.

9 Liu Q, Zhou YH, Yang ZQ. The cytokine storm of severe influenza and development of immunomodulatory therapy. Cell Mol Immunol 2016; 13:3.

10 Klenk HD, Rott R, Orlich M, Blodorn J. Activation of influenza A viruses by trypsin treatment. Virology 1975; 68:426–439.

11 Lazarowitz SG, Choppin PW. Enhancement of the infectivity of influenza A and B viruses by proteolytic cleavage of the hemagglutinin polypeptide. Virology 1975; 68:440–454.

12 Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, *et al.* New world bats harbor diverse influenza A viruses. PLoS Pathog 2013; 9:e1003657.

13 Zhu X, Yu W, McBride R, Li Y, Chen LM, Donis RO, *et al.* Hemagglutinin homologue from H17N10 bat influenza virus exhibits divergent receptor-binding and pH-dependent fusion activities. Proc Natl Acad Sci U S A 2013; 110:1458–1463.

14 Stray SJ, Cummings RD, Air GM. Influenza virus infection of desialylated cells. Glycobiology 2000; 10:649–658.

15 Imai M, Kawaoka Y. The role of receptor binding specificity in interspecies transmission of influenza viruses. Curr Opin Virol 2012; 2:160–167.

16 Nicholls JM, Chan RW, Russell RJ, Air GM, Peiris JS. Evolving complexities of influenza virus and its receptors. Trends Microbiol 2008; 16:149–157.

17 Matrosovic MN, Gambaryan AS, Teneberg S, Piskarev V, Yamnikova SS, Lvov DK, *et al.* Avian influenza A viruses differ from human viruses by recognition of sialyloligosaccharides and gangliosides and by a higher conservation of the HA receptor-binding site. Virology 1997; 233:224–234.

18 Chutinimitkul S, Herfst S, Steel J, Lowen AS, Ye J, van Reil D, *et al.* Virulence-associated substitution D222G in the hemagglutinin of 2009 pandemic influenza A(H1N1) virus affects receptor binding. J Virol 2010; 84:11802–11813.

19 Vines A, Wells K, Matrosovich M, Castrucci MR, Ito T, Kawaoka Y. The role of influenza A virus hemagglutinin residues 226 and 228 in receptor specificity and host range restriction. J Virol 1998; 72:7626–7631.

20 Zhu R, Yang X, Zhang J, Xu D, Fan J, Shi H, *et al.* Identification, sequence analysis, and infectivity of H9N2 avian influenza viruses isolated from geese. J Vet Sci 2018; 19:406–415.

21 Reid AH, Fanning TG, Hultin JV, Taubenberger JK. Origin and evolution of the 1918 'Spanish' influenza virus hemagglutinin gene. Proc Natl Acad Sci U S A 1999; 96:1651–1656.

22 Daniels PS, Jeffries S, Yates P, Schild GC, Rogers GN, Paulson JC, *et al.* The receptor-binding and membrane-fusion properties of influenza virus variants selected using anti-haemagglutinin monoclonal antibodies. EMBO J 1987; 6:1459–1465.

23 Skehel JJ, Wiley DC. Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu Rev Biochem 2000; 69: 531–569.

24 Matrosovich M, Zhou N, Kawaoka Y, Webster R. The surface glycoproteins of H5 influenza viruses isolated from humans, chickens, and wild aquatic birds have distinguishable properties. J Virol 1999; 73:1146–1155.

25 Hardy CT, Young SA, Webster RG, Naeve CW, Owens RJ. Egg fluids and cells of the chorioallantoic membrane of embryonated chicken eggs can select different variants of influenza A (H3N2) viruses. Virology 1995; 211:302–306.

26 Ito T, Suzuki Y, Mitnaul L, Vines A, Kida H, Kawaoka Y. Receptorspecificity of influenza A viruses correlates with the agglutination of erythrocytes from different animal species. Virology 1997; 227:493–499.

27 Wang M, Tscherne DM, McCullough C, Caffrey M, Garcia-Sastre A, Rong L. Residue Y161 of influenza virus hemagglutinin is involved in viral recognition of sialylated complexes from different hosts. J Virol 2012; 86:4455–4462.

28 Munk K, Pritzer E, Kretzschmar E, Gutte B, Garten W, Klenk HD. Carbohydrate masking of an antigenic epitope of influenza virus haemagglutinin independent of oligosaccharide size. Glycobiology 1992; 2:233–240.

29 Skehel JJ, Stevens DJ, Daniels RS, Douglas AR, Knossow M, Wilson IA, Wiley DC. A carbohydrate side chain on hemagglutinins of Hong Kong influenza viruses inhibits recognition by a monoclonal antibody. Proc Natl Acad Sci U S A 1984; 81:1779–1783.

30 Seidel W, Kunkel F, Geisler B, Garten W, Herrmann B, Dohner L, Klenk HD. Intraepidemic variants of influenza virus H3 hemagglutinin differing in the number of carbohydrate side chains. Arch Virol 1991; 120:289–296.

31 Deshpande KL, Fried VA, Ando M, Webster RG. Glycosylation affects cleavage of an H5N2 influenza virus hemagglutinin and regulates virulence. Proc Natl Acad Sci U S A 1987; 84:36–40.

32 Ohuchi M, Orlich M, Ohuchi R, Simpson BE, Garten W, Klenk HD, *et al.* Mutations at the cleavage site of the hemagglutinin after the pathogenicity of influenza virus A/chick/Penn/83 (H5N2). Virology 1989; 168:274–280.

33 Ohuchi R, Ohuchi M, Garten W, Klenk HD. Oligosaccharides in the stem region maintain the influenza virus hemagglutinin in the metastable form required for fusion activity. J Virol 1997; 71:3719–3725.

34 Ohuchi M, Ohuchi R, Feldmann A, Klenk HD. Regulation of receptor binding affinity of influenza virus hemagglutinin by its carbohydrate moiety. J Virol 1997; 71:8377–8384.

35 Byrd-Leotis L, Cummings RD, Steinhauer DA. The interplay between the host receptor and influenza virus hemagglutinin and neuraminidase. Int J Mol Sci 2017; 18:1541.

36 Gambaryan AS, Marinina VP, Tuzikov AB, Bovin NV, Rudneva IA, Sinitsyn BV, *et al.* Effects of host-dependent glycosylation of hemagglutinin on receptor-binding properties on H1N1 human influenza A virus grown in MDCK cells and in embryonated eggs. Virology 1998; 247:170–177.

37 Londrigan SL, Turville SG, Tate MD, Deng YM, Brooks AG, Reading PC. N-linked glycosylation facilitates sialic acid-independent attachment and entry of influenza A viruses into cells expressing DC-SIGN or L-SIGN. J Virol 2011; 85:2990–3000.

38 Feinberg H, Mitchell DA, Drickamer K, Weis WI. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science 2001; 294:2163–2166.

39 Mitchell DA, Fadden AJ, Drickamer K. A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. J Biol Chem 2001; 276:28939–28945.

40 Tisoncik JR, Guo Y, Cordero KS, Yu J, Wang J, Cao Y, *et al.* Identification of critical residues of influenza neuraminidase in viral particle release. Virol J 2011; 8:14.

41 Goto H, Kawaoka Y. A novel mechanism for the acquisition of virulence by a human influenza A virus. Proc Natl Acad Sci U S A 1998; 95:10224–10228.

42 Chaipan C, Kobasa D, Bertram S, Glowacka I, Steffen I, Tsegaye TS, *et al.* Proteolytic activation of the 1918 influenza virus hemagglutinin. J Virol 2009; 83:3200–3211.

43 Lazarowitz SG, Goldberg AR, Choppin PW. Proteolytic cleavage by plasmin of the HA polypeptide of influenza virus: host cell activation of serum plasminogen. Virology 1973; 56:172–180.

44 Ma C, Polishchuk AL, Ohigashi Y, Stouffer AL, Schon A, Magavern E, *et al.* Identification of the functional core of the influenza A virus A/M2 proton-selective ion channel. Proc Natl Acad Sci U S A 2009; 106:12283–12288.

45 Te Velthuis AJ, Fodor E. Influenza virus RNA polymerase: insights into the mechanisms of viral RNA synthesis. Nat Rev Microbiol 2016; 14:479.

46 Schauer R. Achievements and challenges of sialic acid research. Glycoconj J 2000; 17:485–499.

47 Suzuki Y. Gangliosides as influenza virus receptors. Variation of influenza viruses and their recognition of the receptor sialo-sugar chains. Prog Lipid Res 1994; 33:429–457.

48 Gambaryan AS, Tuzikov AB, Piskarev VE, Yamnikova SS, Lvov DK, Robertson JS, *et al.* Specification of receptor-binding phenotypes of influenza virus isolates from different hosts using synthetic sialylglycopolymers: non-egg-adapted human H1 and H3 influenza A and influenza B viruses share a common high binding affinity for 6'-sialyl(N-acetyllactosamine). Virology 1997; 232:345–350.

49 Gambaryan AS, Robertson JS, Matrosovich MN. Effects of egg-adaptation on the receptor-binding properties of human influenza A and B viruses. Virology 1999; 258:232–239.

50 Varki A. Uniquely human evolution of sialic acid genetics and biology. Proc Natl Acad Sci U S A 2010; 107(Suppl 2):8939–8946.

51 Gagneux P, Cheriyan M, Hurtado-Ziola N, van der Linden EC, Anderson D, McClure H, *et al.* Human-specific regulation of alpha 2-6-linked sialic acids. J Biol Chem 2003; 278:48245–48250.

52 Higa HH, Rogers G, Paulson JC. Influenza virus hemagglutinins differentiate between receptor determinants bearing N-acetyl-, N-glycollyl-, and N,O-diacetylneuraminic acids. Virology 1985; 144:279–282.

53 Ichikawa S, Nakajo N, Sakiyama H, Hirabayashi Y. A mouse B16 melanoma mutant deficient in glycolipids. Proc Natl Acad Sci U S A 1994; 91:2703–2707.

54 Chu VC, Whittaker GR. Influenza virus entry and infection require host cell N-linked glycoprotein. Proc Natl Acad Sci U S A 2004; 101:18153–18158.

55 Kang MS, Elbein AD. Alterations in the structure of the oligosaccharide of vesicular stomatitis virus G protein by swainsonine. J Virol 1983; 46:60–69.

56 Connor RJ, Kawaoka Y, Webster RG, Paulson JC. Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. Virology 1994; 205:17–23.

57 Ito T, Couceiro JN, Kelm S, Baum LG, Krauss S, Castrucci MR, *et al.* Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J Virol 1998; 72:7367–7373.

58 Couceiro JN, Paulson JC, Baum LG. Influenza virus strains selectively recognize sialyloligosaccharides on human respiratory epithelium; the role of the host cell in selection of hemagglutinin receptor specificity. Virus Res 1993; 29:155–165.

59 Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, *et al.* Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 1998; 279:393–396.

60 Claas EC, Osterhaus AD, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, *et al.* Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 1998; 351:472–477.

61 Matrosovich MN, Matrosovich TY, Gray T, Roberts NA, Klenk HD. Human and avian influenza viruses target different cell types in cultures of human airway epithelium. Proc Natl Acad Sci U S A 2004; 101:4620–4624.

62 Nicholls JM, Bourne AJ, Chen H, Guan Y, Peiris JS. Sialic acid receptor detection in the human respiratory tract: evidence for widespread distribution of potential binding sites for human and avian influenza viruses. Respir Res 2007; 8:73.

63 Horimoto T, Kawaoka Y. Pandemic threat posed by avian influenza A viruses. Clin Microbiol Rev 2001; 14:129–149.

64 Gambaryan AS, Tuzikov AB, Pazynina GV, Desheva JA, Bovin NV, Matrosovich MN, *et al.* 6-sulfo sialyl Lewis X is the common receptor determinant recognized by H5, H6, H7 and H9 influenza viruses of terrestrial poultry. Virol J 2008; 5:85.

65 Glaser L, Conenello G, Paulson J, Palese P. Effective replication of human influenza viruses in mice lacking a major alpha2,6 sialyltransferase. Virus Res 2007; 126:9–18.

66 Gambarian AS, Marinina VP, Solodar TA, Bovin NV, Tuzikov AB, Pazynina GV, *et al.* Different receptor specificity of influence viruses from ducks and chickens and its reflection in the composition of sialosides on host cells and mucins. Vopr Virusol 2006; 51:24–32.

67 Wan H, Perez DR. Quail carry sialic acid receptors compatible with binding of avian and human influenza viruses. Virology 2006; 346:278–286.

68 Vidaña B, Dolz R, Busquets N, Ramis A, Sánchez R, Rivas R, *et al.* Transmission and immunopathology of the avian influenza virus A/Anhui/1/2013 (H7N9) human isolate in three commonly commercialized avian species. Zoonoses Public Health 2018; 65:312–321.

69 Ibricevic A, Pekosz A, Walter MJ, Newby C, Battaile JT, Brown EG, *et al.* Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. J Virol 2006; 80:7469–7480.

70 Kogure T, Suzuki T, Takahashi T, Miyamoto D, Hidari KI, Guo CT, *et al.* Human trachea primary epithelial cells express both sialyl(alpha2-3)Gal receptor for human parainfluenza virus type 1 and avian influenza viruses, and sialyl(alpha2-6)Gal receptor for human influenza viruses. Glycoconj J 2006; 23:101–106.

71 Rogers GN, Pritchett TJ, Lane JL, Paulson JC. Differential sensitivity of human, avian, and equine influenza A viruses to a glycoprotein inhibitor of infection: selection of receptor specific variants. Virology 1983; 131:394–408.

72 Rogers GN, Paulson JC, Daniels RS, Skehel JJ, Wilson IA, Wiley DC. Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. Nature 1983; 304:76–78.

73 Gambaryan AS, Matrosovich TY, Boravleva EY, Lomakina NF, Yamnikova SS, Tuzikov AB, *et al.* Receptor-binding properties of influenza viruses isolated from gulls. Virology 2018; 522:37–45.

74 de Graaf M, Fouchier RA. Role of receptor binding specificity in influenza A virus transmission and pathogenesis. EMBO J 2014; 33:823–841.

75 Gambaryan AS, Tuzikov AB, Bovin NV, Yamnikova SS, Lvov DK, Webster RG, *et al.* Differences between influenza virus receptors on target cells of duck and chicken and receptor specificity of the 1997 H5N1 chicken and human influenza viruses from Hong Kong. Avian Dis 2003; 47:1154–1160.

76 Gambaryan A, Tuzikov A, Pazynina G, Bovin N, Balish A, Klimov A. Evolution of the receptor binding phenotype of influenza A (H5) viruses. Virology 2006; 344:432–438.

77 Bewley CA. Illuminating the switch in influenza viruses. Nat Biotechnol 2008; 26:60–62.

78 Xu D, Newhouse EI, Amaro RE, Pao HC, Cheng LS, Markwick PR, *et al.* Distinct glycan topology for avian and human sialopentasaccharide receptor analogues upon binding different hemagglutinins: a molecular dynamics perspective. J Mol Biol 2009; 387:465–491.

79 Nicholls JM, Chan MC, Chan WY, Wong HK, Cheung CY, Kwong DL, *et al.* Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. Nat Med 2007; 13:147–149.

80 Bandlow V, Liese S, Lauster D, Ludwig K, Netz RR, Herrmann A, *et al.* Spatial screening of hemagglutinin on Influenza A virus particles: Sialyl-LacNAc displays on DNA and PEG scaffolds reveal the requirements for bivalency enhanced interactions with weak monovalent binders. J Am Chem Soc 2017; 139:16389–16397.

81 Kage A, Shoolian E, Rokos K, Ozel M, Nuck R, Reutter W, *et al.* Epithelial uptake and transport of cell-free human immunodeficiency virus type 1 and gp120-coated microparticles. J Virol 1998; 72:4231–4236.

82 Soilleux EJ. DC-SIGN (dendritic cell-specific ICAM-grabbing non-integrin) and DC-SIGN-related (DC-SIGNR): friend or foe? Clin Sci (Lond) 2003; 104:437–446.

83 Lin G, Simmons G, Pohlmann S, Baribaud F, Ni H, Leslie GJ, *et al.* Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. J Virol 2003; 77:1337–1346.

84 Tassaneetrithep B, Burgess TH, Granelli-Piperno A, Trumpfheller C, Finke J, Sun W, *et al.* DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 2003; 197:823–829.

85 Han DP, Lohani M, Cho MW. Specific asparagine-linked glycosylation sites are critical for DC-SIGN- and L-SIGN-mediated severe acute respiratory syndrome coronavirus entry. J Virol 2007; 81:12029–12039.

86 Marzi A, Gramberg T, Simmons G, Moller P, Rennekamp AJ, Krumbiegel M, *et al.* DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. J Virol 2004; 78:12090–12095.

87 Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, *et al.* DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 2000; 100:587–597.

88 Snyder GA, Ford J, Torabi-Parizi P, Arthos JA, Schuck P, Colonna M, *et al.* Characterization of DC-SIGN/R interaction with human immunodeficiency virus type 1 gp120 and ICAM molecules favors the receptor's role as an antigen-capturing rather than an adhesion receptor. J Virol 2005; 79:4589–4598.

89 Davis CW, Nguyen HY, Hanna SL, Sanchez MD, Doms RW, Pierson TC. West Nile virus discriminates between DC-SIGN and DC-SIGNR for cellular attachment and infection. J Virol 2006; 80:1290–1301.

90 Lozach PY, Lortat-Jacob H, Lacroix de Lavalette A, Staropoli I, Foung S, Amara A, *et al.* DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. J Biol Chem 2003; 278:20358–20366.

91 Chen Y, Chan VS, Zheng B, Chan KY, Xu X, To LY, *et al.* A novel subset of putative stem/progenitor CD34+Oct-4+ cells is the major target for SARS coronavirus in human lung. J Exp Med 2007; 204:2529–2536.

92 Jeffers SA, Tusel SM, Gillim-Ross L, Hemmila EM, Achenbach JE, Babcock GJ, *et al.* CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci U S A 2004; 101:15748–15753.

93 Chan MC, Chan RW, Yu WC, Ho CC, Chui WH, Lo CK, *et al.* Influenza H5N1 virus infection of polarized human alveolar epithelial cells and lung microvascular endothelial cells. Respir Res 2009; 10:102.

94 Sumikoshi M, Hashimoto K, Kawasaki Y, Sakuma H, Suzutani T, Suzuki H, *et al.* Human influenza virus infection and apoptosis induction in human vascular endothelial cells. J Med Virol 2008; 80:1072–1078.

95 Fujioka Y, Nishide S, Ose T, Suzuki T, Kato I, Fukuhara H, *et al.* A sialylated voltage-dependent Ca2+ channel binds hemagglutinin and mediates influenza A virus entry into mammalian cells. Cell Host Microbe 2018; 23:809–818.

96 Tailleux L, Schwartz O, Herrmann JL, Pivert E, Jackson M, Amara A, *et al.* DC-SIGN is the major Mycobacterium tuberculosis receptor on human dendritic cells. J Exp Med 2003; 197:121–127.

97 Van Pottelberge GR, Bracke KR, Demedts IK, De Rijck K, Reinartz SM, van Drunen CM, *et al.* Selective accumulation of langerhans-type dendritic cells in small airways of patients with COPD. Respir Res 2010; 11:35.

98 Guo Y, Feinberg H, Conroy E, Mitchell DA, Alvarez R, Blixt O, *et al.* Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. Nat Struct Mol Biol 2004; 11:591–598.

99 Kumari K, Gulati S, Smith DF, Gulati U, Cummings RD, Air GM. Receptor binding specificity of recent human H3N2 influenza viruses. Virol J 2007; 4:42.

100 Mogensen SC. Role of macrophages in natural resistance to virus infections. Microbiol Rev 1979; 43:1–26.

101 Wijburg OL, DiNatale S, Vadolas J, van Rooijen N, Strugnell RA. Alveolar macrophages regulate the induction of primary cytotoxic T-lymphocyte responses during influenza virus infection. J Virol 1997; 71:9450–9457.

102 Cheung CY, Poon LL, Lau AS, Luk W, Lau YL, Shortridge KF, *et al.* Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? Lancet 2002; 360:1831–1837.

103 Perrone LA, Plowden JK, Garcia-Sastre A, Katz JM, Tumpey TM. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. PLoS Pathog 2008; 4:e1000115.

104 Rodgers BC, Mims CA. Influenza virus replication in human alveolar macrophages. J Med Virol 1982; 9:177–184.

105 Rodgers B, Mims CA. Interaction of influenza virus with mouse macrophages. Infect Immun 1981; 31:751–757.

106 Pontow SE, Kery V, Stahl PD. Mannose receptor. Int Rev Cytol 1992; 137B:221–244.

107 Reading PC, Miller JL, Anders EM. Involvement of the mannose receptor in infection of macrophages by influenza virus. J Virol 2000; 74:5190–5197.

108 Upham JP, Pickett D, Irimura T, Anders EM, Reading PC. Macrophage receptors for influenza A virus: role of the macrophage galactose-type lectin and mannose receptor in viral entry. J Virol 2010; 84:3730–3737.

109 Chen X, Liu S, Goraya MU, Maarouf M, Huang S, Chen JL. Host immune response to influenza A virus infection. Front Immunol 2018; 9:320.

110 Kimura T, Imai Y, Irimura T. Calcium-dependent conformation of a mouse macrophage calcium-type lectin. Carbohydrate binding activity is stabilized by an antibody specific for a calcium-dependent epitope. J Biol Chem 1995; 270:16056–16062.