Review

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Review

Knockin' on Cell's Door: Influenza A Virus Adsorption and its Pharmacological Inhibition

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Abstract: Influenza A virus (IAV) is a widespread human respiratory pathogen that contributes significantly to morbidity and mortality worldwide. Adsorption of the virus to the cell surface is the earliest stage of its replication cycle. The key role of N-linked sialic acids as receptors for binding to IAV's hemagglutinin has long been acknowledged. The molecular specificity of this interaction is a key factor in host range, pathogenicity and transmissibility of various IAV subtypes. Along with that, a number of recent studies have introduced significant complexity into the picture of the IAV adsorption and revealed a multitude of new molecules on host cell surface to serve as receptors and/or co-receptors for IAV attachment. For successful internalization of the adsorbed virus, a downstream signal transduction is necessary to activate effector endocytosis mechanisms. In recent years, our understanding of the sophistication and variability of signal transduction pathways in the virus attachment site has significantly expanded, with the help of such research techniques as fluorescence imaging of individual viruses in real time, dominant-negative mutants, siRNA knockdowns, protein kinase selective inhibitors, phosphoproteome profiling, and others. These approaches deepen our knowledge of the molecules involved in the early stages of the IAV life cycle, and also serve as the basis for the development of new effective antiviral drugs. In our review, we analyze recent publications on the mechanisms of IAV adsorption, newly discovered receptors for virus attachment, and signal transmission in the site of the adsorbed virion. Besides, we consider new data on the development of selective inhibitors as antiviral drugs aimed at both viral and cellular factors of IAV adsorption.

Keywords: influenza A virus; hemagglutinin; neuraminidase; sialic acid; cell receptors; signal transduction; selective inhibitors; drug development

1. Introduction.

Influenza A virus is a widespread human respiratory pathogen which evokes both single cases and local outbreaks of the disease, as well as massive seasonal epidemics and pandemics. Circulating influenza A viruses annually lead to serious medical and socio-economic consequences [1]. Worldwide, these epidemics are estimated to cause between 3 and 5 million cases of severe illness and between 290 000 and 650 000 deaths each year [2].

Influenza A virus belongs to the Orthomyxoviridae family, is enveloped and characterized by segmented, single-stranded and negative-sense RNA genome [3]. Eight genomic IAV's segments encode 10–18 proteins, depending on the virus strain [4]. Among these viral proteins, the surface glycoproteins hemagglutinin (encoded by segment 4) and neuraminidase (encoded by segment 6) are key players in the adsorption of the virus, and also represent the predominant targets for neutralizing antibodies.

At all stages of the replication cycle, IAV interacts closely with a variety of effector and signaling systems of the host cell, hijacking numerous cellular functions for its own reproduction and reorganizing them for the smooth passage of the life cycle [5,6]. Meanwhile, the plasma membrane represents the main obstacle to be overcome by the enveloped IAV for successful infection of the cell,

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and internalization of a virus through the plasma membrane barrier requires a complex, space- and time-coordinated combination of viral and cellular protein machinery [7,8].

Adsorption of the virus is the starting point of its reproduction cycle. Basically, it is determined by the interaction of viral hemagglutinin with N-acetylneuramic (sialic) acid (SIA) on the surface of the target cell. In addition, in recent years a number of new data have been accumulated that significantly expand our understanding of the repertoire of receptors available for the IAV attachment to the cell surface. These new receptors include both sialylated and non-sialylated membrane proteins and lipids, and interaction with them during viral attachment involves both hemagglutinin and neuraminidase.

It should be noted that the attachment of the virus to the plasma membrane does not ensure its automatic endocytosis. The initiation of endocytosis requires a variety of intracellular signaling events, mainly phosphorylation cascades, to transmit a signal from receptor proteins to effector systems of endocytosis machinery [9].

Studies of signaling pathways preceding an IAV endocytosis require the synthesis of classical virology, cellular, molecular and structural biology methods. Among them, dominant-negative mutants, siRNA and selective inhibitor screens, phosphoproteomics should be highlighted as the most informative techniques. Besides, direct visualization of adsorption, endocytosis, and their individual components is of especial importance. Essentially, transmission electron microscopy (TEM) remains the only method for detailed visualization of viral particles and cell proteins with the nano-scale resolution, though it does not allow exploring living objects [10]. Studies of the dynamics of virus attachment and internalization inevitably require the use of multicolor time-lapse confocal microscopy, with fluorescent labeling of viruses and overexpression of fluorescent proteins of interest involved in signaling processes [11,12]. Techniques of ultra-high-resolution optical microscopy allow to visualize localization and dynamics of viruses at the level of individual viral particles and even individual molecules and, in principle, approach the resolution of an electron microscope [13].

Early stages of IAV infection are an attractive target for new chemotherapeutic intervention strategies [14,15]. Significantly, key cellular factors are promising targets for the development of new generation antiviral drugs, as an alternative to rapidly mutating viral target proteins [16]. Therefore, the most in-depth knowledge of the mechanisms of IAV reception and signal transduction, in addition to a better understanding of the fundamentals of infection, is of great practical interest for the development of novel approaches to the prevention and therapy of influenza.

Our review concerns the recent data on the cellular mechanisms of the IAV adsorption by the host cell. We consider the receptor mechanisms involved, analyze recent results on the signaling cascades at the IAV attachment point. We pay special attention to the selective inhibitors of viral and cellular factors of IAV adsorption as the important research tool in the cell biology of IAV infection, and as new candidate anti-viral therapeutical agents.

2. Influenza A Virus Adsorption.

The lipoprotein envelope of the influenza A virus contains spikes of two viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA). To date, 18 subtypes of HA (H1-H18) and 11 subtypes of NA (N1-N11) are known, corresponding to a variety of IAV subtypes. Of them, H1N1 and H3N2 viruses circulate in the human population. Also, sporadic zoonotic humans' infections by viruses of different subtypes, such as H5N1, H7N9, and others, were registered. These zoonotic infections are of especial concern, since they are often accompanied by a severe course of the disease and a relatively high mortality rate [17].

HA is a receptor-binding protein that mediates the attachment of viral particles to the host cell surface. The adsorption of the virus on the plasma membrane basically depends on the interaction of the H1 subunit of viral hemagglutinin with N-acetylneuramic (sialic) acid (SIA) on the surface of the target cell. The molecular specificity of this interaction is an extremely significant (but not the only) factor in viral tropism, pathogenicity and transmissibility of various IAV subtypes and strains and is being actively studied [18–20].

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On the cell surface, SIAs are linked to either N-glycan chains, which are asparagine bound, or O-glycan chains, which are serine or threonine bound. Typically, N-glycan bound sialic acids are considered to be the main hemagglutinin – interacting moieties. In general, hemagglutinins of IAV strains infecting humans preferentially bind to 2,6-linked SIAs (SIA C2 - galactose C6 linkage), while avian strains recognize 2,3-linked SIAs (SIA C2 - galactose C3 linkage) [21,22], though zoonotic transmission of infection can periodically break this rule [23]. A number of hemagglutinin point mutations were reported which can lead to a change in the IAV host from birds to humans [24,25]. In addition to the SIA terminal bond, the topology of the glycan itself (long umbrella-shaped or short cone-shaped glycan structure) makes a significant contribution to the receptor specificity of hemagglutinin [26].

The second IAV surface protein, neuraminidase, is an enzyme which ensures cleavage of α-ketosidic linkage between the SIA and an adjacent sugar [27]. This function is vital for the successful budding completion and release of viral progeny from the surface of an infected cell [28]. Along with this, the activity of neuraminidase plays an equally important role in the early stages of the IAV life cycle. It is known that gel-forming mucins of the respiratory tract are the decoy receptors for influenza virions. Binding of virions to SIAs of these decoys with their subsequent elimination via the mucociliary clearance diminishes infection of respiratory epithelial cells. SIAs cleavage by NA facilitates the release of viral particles from decoy receptors in mucus of respiratory tract and helps the virions reach the target epithelial cells [29,30].

Another recently established important function of NA sialidase activity at the reception stage is to ensure lateral mobility of the adsorbed virion along the cell surface (virion rolling) for its movement to the membrane site with pronounced endocytic activity [31,32]. This receptor gradient-dependent virion rolling is realized as a synergistic effect of the receptor function of HA and the sialidase activity of NA in the area of attachment [33]. In general, the functional balance between HA/NA activities largely affects the infectivity, pathogenicity and adaptation of IAV to the host [34,35].

N-glycan bound sialic acids are not the only receptor for hemagglutinin of the influenza virus. De Vries and co-authors demonstrated that H1N1 IAV is able to infect cells of several lines deficient in N-acetylglucosamine transferase 1 (GnT1) and completely devoid of sialylated N-linked glycans [36]. According to the authors, in GnT1- deficient cells the virus internalization nevertheless remained SIA-dependent, which suggests an important role of other receptors, such as sialylated O-linked glycans, in the entry of the virus [36].

In addition to that, there is repeated evidence in the literature that IAV is able to infect cells with completely enzymatically removed SIAs [37,38]; hence IAV attachment to the host cell may occur SIA-independently.

Noteworthy, nonsialylated phosphoglycans were found to be an alternative molecular target for hemagglutinin, with the binding site different from the canonical HA receptor-binding domain [39]. These results suggest that IAV's hemagglutinin is capable of coupling both sialoglycans and phosphoglycans on the cell surface, and has two separate binding sites for these interactions.

Recent studies have also specified several cell proteins, which are capable of binding to IAV's glycoproteins, HA and NA. These proteins include the Immunomodulatory CEA Cell Adhesion Molecule 6 (CEACAM6/CD66c) [40]. The authors demonstrated that CD66c serves as a cellular receptor for IAV, by binding to the neuraminidase of the virus. Overexpression of CD66c in A549 cells significantly increases infection, while blocking of this protein by antibodies or its siRNA-knockdown inhibits virus entry into cells.

In another study, it was shown that the pattern-recognizing receptor TLR4 expressed on the surface of target cells binds to high mannose residues on the virus surface glycoproteins during the virus adsorption [41]. It is well known that TLR4-based signaling is an essential component of innate immunity response. At the same time, it is believed that the influenza virus has evolved in such a way to exploit this pathway to facilitate its entry into an infected cell [41].

In addition, several members of the calcium-dependent C-type lectins family were demonstrated to interact with IAV's glycoproteins. These lectins include macrophage mannose receptor (MMR)

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[42,43], macrophage galactose-type lectin 1 (MGL1) [43,44], langerin [45], DC-SIGN (CD209) [46–49], and L-SIGN (CD209L) [47,49]. The listed calcium-dependent C-type lectins are expressed in cells of the immune system, such as macrophages and dendritic cells, excluding L-SIGN, which is a primarily endothedial protein. It has long been known that the IAV is capable of infecting cells of these types [50–52]. Though such an infection is abortive, it stimulates the secretion of multitude proinflammatory cytokines by these immune cells to orchestrate an anti-viral response in lungs [53]. The above-mentioned macrophages' and DCs' lectins recognize N-linked glycans of viral glycoproteins, and apparently are able to bind to both hemagglutinin and neuraminidase glycans [54].

Additional cellular proteins were shown to be partners in coupling to HA of some specific IAV subtypes. Particularly, it was revealed that the H17N10 and H18N11 bat viruses do not bind SIAs [55,56]. Instead, they enter target cells via the main histocompatibility complex (MHC) class II coupling [57]. More recent data demonstrated that H2N2 viruses can bind MHC II, too, with dual receptor specificity of H2 hemagglutinin for SIAs and MHC class II [58]. Currently, there is no deeper information on the possible cofactors of this binding and on signaling pathways mediating MHC II - dependent internalization of the virus.

Thus, according to the sum of available data, sialylated N-glycan binding is the predominant way of IAV adsorption on the membranes of the respiratory tract epithelia. At the same time, IAV receptor specificity is not limited exclusively to sialic acids and involves several additional host cell protein factors and/or co-factors. Besides receptor specificity, the shape of virions is an essential factor in their successful attachment to the cell membrane. As known, virions of different strains can have various shapes, either rounded, with an average diameter of 120-150 nm, or filamentous, reaching a length of 10 microns or more. The genetic determinant of the assembly of virions with rounded or filamentous morphology is encoded by the M1 gene sequence. In addition, some cellular factors affect the shape of viral particles budding on the plasma membrane [59,60]. According to the recently published results, under pressure from the host's immune system, which tends to neutralize HA and NA, long filamentous virions containing multiple surface glycoproteins have a higher probability of attachment and further internalization compared to small rounded viral particles [61]. Even if 95% of its glycoproteins are compromised, filamentous virion can be successfully adsorbed and internalized [61].

3. Signal Transduction at the IAV Adsorption Site.

Sialic acids on the apical surface of the epithelial cell are present as N-glycan bound and O-glycan bound, being a part of either glycolipids or glycoproteins. Apparently, IAV's HA can provide initial attachment of the virus via SIA-coupling in any case, since hemagglutinin effectively binds to sialoglycans in vitro in the absence of cellular proteins or lipids, as was demonstrated by Weis and co-authors' structural biology data [62].

However, by themselves, SIAs are not able to perform the downstream signaling for subsequent endocytosis machinery activation. In recent years, the efforts of researchers have been focused on figuring out which cellular factors are involved in the signal transduction in the earliest stages of IAV entry. As a result, several cellular membrane hemagglutinin binding proteins have been reported to be involved in subsequent initiation of IAV endocytosis.

These signal-transmitting proteins include the EGF receptor (EGFR), a well-researched receptor tyrosine kinase abundantly expressed in the respiratory epithelia. Several recent studies found that EGFR binds to IAV in a SIA-dependent manner, what triggers the EGFR tyrosine autophosphorylation activity, with following activation of several downstream pathways such as PI3K/Akt pathway [63], FAK pathway [64], PLC-1 signaling [65], which in turn trigger the protein machinery of endocytosis. The involvement of the listed signaling pathways in launching of IAV endocytosis has been shown using a number of inhibitors such as genistein (the broad-range tyrosine kinase inhibitor [63], anti-EGFR siRNA [62], FAK inhibitor I [64], U73122 (selective PLC-γ 1 inhibitor) [65], anti-PLC-γ 1 shRNA [65], M85 (EGFR and PIK3C2β inhibitor) [66], which significantly reduced IAV internalization in the above works.

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The key role of EGFR in IAV internalization was further confirmed by bioinformatics, including integration transcriptome/proteome clustering and association network topology analysis [67]. Authors of this publication specify EGFR as bottleneck regulator with corroborating signals across transcript and protein expression data. They also provide experimental in vivo evidence of EGFR significance in IAV infection using gefitinib pharmacological inhibition of EGFR on the mouse model of infection [67].

Importantly, fluorescent microscopy revealed the presence of cholesterol-enriched lipid rafts co-localized with attached IAV virions and EGFR, as local areas of downstream signal transduction [63]. A recent data from Sieben and co-authors obtained by ultra-high-resolution fluorescent STED and STORM imaging revealed the pre-formed EGFR clusters that existed before interaction with the virus particle [68]. It means that there are specialized endocytic active membrane domains that must be reached by lateral rolling of IAV virion for its further successful internalization.

The voltage-dependent Ca2+ channel $Ca_v1.2$ has been reported to be another protein mediating signal transduction in the virion attachment region. This type of calcium channel is widespread in various tissues of the body, including myocardium, brain, smooth muscles of various localization, and lungs. Similar to EGFR, $Ca_v1.2$ is sialylated and binds to IAV's hemagglutinin [69]. $Ca_v1.2$ binding to the influenza A virus promotes the influx of calcium into the cytoplasm and stimulates calcium oscillations, as shown by using the FRET-based Ca2+ sensor YC3.60. On the contrary, pharmacological $Ca_v1.2$ inhibition by calcium channel blockers, especially by diltiazem, or siRNA-based $Ca_v1.2$ knockdown leads to a significant decrease in cell infection by IAV and, at the same time, to the cessation of calcium oscillations [70]. The key role of calcium influx for the successful entry of the influenza virus has been repeatedly noted [69,70].

Details of the downstream Ca^{2+}- mediated signaling following an IAV attachment were analyzed by Fujioka and co-authors [71]. The authors reported two axes of calcium-dependent signaling at the IAV entry, namely, RhoA-ROCK-PIP5K pathway leading to the clathrin-mediated endocytosis (CME) activation, and also Ras-PI3K-Rac1 pathway to stimulate clathrin-independent endocytosis (CIE), presumably macropinocytosis. Importantly, it is assumed that the CME and CIE pathways are redundant, and internalization of every viral particle occurs in a cell context- and condition-dependent manner [71].

As mentioned above, TLR4 has been reported to bind mannose-rich glycans of IAV glycoproteis [41]. In this article, Marchant et al. provided microscopic evidence of TLR4 clustering in tight co-localization with attached viral particles. They also demonstrated fast activation of the p38-MAPK signaling at the site of IAV-TLR4 interaction. When p38-MAPK was inhibited by selective inhibitor SB203580, a reduction in virus entry was observed [41].

In addition to the aforementioned, there are several other proteins that bind to hemagglutinin and are possible factors (or cofactors) of IAV adsorption and further downstream signal transduction. These include nucleolin, a widespread protein with multiple functions and interaction partners. In a cell, nucleolin is localized both in the nucleus and in the cytoplasm. In the nucleus of an IAV infected cell, nucleolin interacts with the viral proteins NS1 [72], NP [73] and PA [74], performing multiple functions in the later stages of the virus reproductive cycle. Meanwhile, at the cell surface nucleolin binds IAV's HA, and siRNA knockdown of nucleolin significantly reduces viral entry into A549 cells [75]. The authors of the article associate this interaction with the role of nucleolin in CME of IAV, although the details remain unspecified.

Recently, Mazel-Sanchez et al. identified transferrin receptor 1 (TfR1) as one more candidate entry protein for IAV, using gain-of-function and loss-of-function genetic experiments as well as in vitro and in vivo chemical inhibition [76]. In respiratory tissue, TfR1 is expressed on macrophages and on the apical side of type I and II pneumocytes, and continuously circulates between the cell surface and the endosome. Mazel-Sanchez et al. showed that TfR1 recycling is vital for its role in IAV entry, by use of recycling-deficient TfR1 mutant [76]. Also, they showed by TIRF microscopy the tight co-localization of TfR1 with internalized fluorescently labeled IAV virus-like particles.

In another recent study, metabotropic glutamate receptor subtype 2 (mGluR2), a G-protein-coupled receptor, has been reported to bind hemagglutinin of several IAV subtypes, H1, H5 and H7

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[77]. Using a variety of techniques such as siRNA knockdown, STED super-resolution microscopy, immunoelectron microscopy, endocytosis inhibitors, and others, Ni and co-authors demonstrated that mGluR2 serves as a cellular receptor for hemagglutinin. This interaction is of high significance for the IAV clathrin-mediated endocytosis, with the key role of the potassium calcium-activated channel subfamily M alpha 1 (KCa1.1) in the downstream signal transduction for the following F-actin polymerization necessary to complete the CME [77].

Similarly, free fatty acid receptor 2 (FFAR2), also being a G-protein-coupled receptor, was demonstrated to be an important cofactor for IAV entry into host cells [78]. According to this work, siRNA knockdown or chemical inhibition of FFAR2 blocks IAV internalization. The authors found that the FFAR2 binds to the hemagglutinin H1 subunit, and is phosphorylated by G protein-coupled receptor kinases, primarily GRK6. The subsequent transmission of the signal for the initiation of CME involves β-arrestin1–AP2B1 signaling cascade [78].

In addition to such cell targets of IAV infection as epithelial cells of the upper and lower respiratory tract, macrophages and dendritic cells, one more cell type known to bind IAV virions is natural killer cells (NKs). From the abundant repertoire of NK cell receptors, natural cytotoxicity receptors NKp46 and NKp44 were repeatedly demonstrated to bind HA of various subtypes [79–81]. NKp46 and NKp44 coupling to HA is SIA-dependent. Evidence of the involvement of both N-linked and O-linked sialylated glycans in the reception has been reported [82–84].

To our best knowledge, currently there is no direct data published on the pathways of downstream signal transduction in NK from NCRs at IAV infection. However, in general, signal transmission from activated NCRs has been investigated previously. It involves Syk/Zap70-PI3K-Rac-PAK-MEK-ERK signaling which finally results in NK cell degranulation and release of multiple cytokines, as well as perforin and granzyme B to mediate NKs' cytotoxic effect [85]. In context of influenza, NKs' cytotoxic action eliminates IAV-infected respiratory epitheliocytes. At the same time, NKs' infection with highly pathogenic IAV strains promotes apoptosis of these innate immunity cells and worsens the course of the disease [86].

The published data on the host cell proteins binding to IAV upon its attachment and on the downstream signaling pathways involved are summarized in the Table 1.

Table 1. Host cell protein receptors/co-receptors for IAV binding and downstream signaling pathways involved.

Receptor/co-receptor protein for IAV binding	Protein normal function in the cell	Downstream signaling upon IAV adsorption	References
CEA Cell Adhesion Molecule 6 (CEACAM6/CD66c)	Regulation of cell adhesion	ns**	40
TLR4 (CD284)	PRR*, activation of innate immune response	p38-MAPK	41
Macrophage mannose receptor (MMR)	PRR, activation of immune response	ns	42, 43
Macrophage galactose-type lectin 1 (MGL1)	PRR, activation of immune response	ns	43, 44
Langerin	PRR, activation of immune response	ns	45
DC-SIGN (CD209)	PRR, activation of immune	ns	46-49

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	response		
L-SIGN (CD209L)	PRR, activation of immune response	ns	47, 49
Main histocompatibility complex class II (MHC II)	Antigen presentation	ns	57, 58
EGF receptor (EGFR)	Stimulation of epithelial cell growth and differentiation	PI3K/Akt FAK PLC-1	63 64 65 67, 68
Voltage-dependent Ca^{2+} channel $Ca_v1.2$	Transmembraneous Ca^{2+} influx	RhoA-ROCK-PIP5K Ras-PI3K-Rac1	69, 70
Nucleolin	Decondensation of intranucleolar chromatin	ns	75
Transferrin receptor 1 (TfR1)/CD71	Transport of iron into the cell	ns	76
Metabotropic glutamate receptor subtype 2 (mGluR2)	Modulation of the synaptic transmission in the CNS	Potassium calcum-activated channel subfamily M alpha 1 (KCa1.1)	77
Free fatty acid receptor 2 (FFAR2)	Regulation of metabolic homeostasis	β-arrestin1–AP2B1	78
NKp46 and NKp44	Activation of NK's cytotoxic effect	ns	79-81

*PRR – pattern recognizing receptor, **ns – not specified.

Thus, to date, a number of both epithelial and non-epithelial cell surface proteins have been proposed as signal-transmitting receptors for the initiation of IAV endocytosis. Despite the close attention of the research community, precise roles of these receptor proteins and especially the hypothetical functional interplay between them in the context of IAV internalization still remain elusive. Apparently, when infecting a cell, IAV has a number of opportunities for receptor binding and several signaling cascades for the launching of endocytosis, and the triggered endocytic pathways turn out to be different, both clathrin-dependent and clathrin-independent. In addition, the shape of viral particles is of importance for the pathway of their endocytosis. According to immunofluorescence and electron microscopy data, IAV strains forming long filamentous virions up to 10 microns in length, such as A/Udorn/72 (H3N2), are endocytosed by macropinocytosis, without significant participation of CME [87], while in the case of rounded virions, about 60% of them are internalized via CME, while the remaining 40% penetrate via CIE [88].

4. Inhibitors of IAV Adsorption in Research and Therapy.

Currently, cell physiology and molecular medicine have in their arsenal a huge number of selective inhibitors of cellular functions and individual molecules. These inhibitors are represented by compounds of a different nature, such as small molecules, peptides, inhibiting antibodies. They are widely used in research, including in most above-referenced publications analyzing the adsorption of the influenza A virus. Functional dissection of cellular processes in viral infection by

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selective inhibitors, particularly by such informative high throughput techniques as protein kinase inhibitor library screen [89], as well as genome-wide siRNA screen [90] or CRISPR screen [91] is a very advantageous research strategy. This approach has revealed a number of novel cellular proteins to be involved in virus replication, for further in-depth analysis. Selective inhibitors of host cell factors exhibiting a pronounced reduction of viral replication represent a powerful tool in studying the molecular aspects of viral pathogenesis and, at the same time, straight come into focus of attention as potential antiviral drugs. In terms of drug development, the adsorption of the influenza virus, as the earliest stage of its replicative cycle, is actively investigated for the possibility of pharmacological inhibition. Researchers consider both viral and cellular factors of viral adsorption as candidate molecular targets.

4.1. Pharmacological Inhibition of Viral Factors of IAV Adsorption.

IAV's surface glycoproteins NA and HA are key factors of adsorption and are being actively investigated as targets for inhibition. Neuraminidase inhibitors (NAIs) are a group of globally approved etiotropic anti-influenza drugs that are most actively marketed worldwide. Currently, this group includes four drugs, namely, zanamivir, oseltamivir, peramivir and laninamivir. Of them, zanamivir and peramivir are administered in active form, while oseltamivir and laninamivir are prodrugs which need to be metabolized in the body into the active agent. Namely, oseltamivir is administered orally as oseltamivir phosphate salt which is hydrolyzed into active oseltamivir carboxylate by hepatic esterases. Laninamivir formulation is registered in Japan. The prodrug laninamivir octanoate is administered as an inhalation powder and is hydrolyzed in lungs [92]. Mechanistically, NAIs bind to the NA catalytic center, thereby blocking its enzymatic activity [28]. In general, the frequency of occurrence of IAV strains resistant to NAIs is estimated as relatively low, although they are regularly reported to be found in circulation [93].

As discussed above, NA plays a significant role both at the earliest and at the latest stages of the IAV replicative cycle. In 2006, Ohuchi and co-authors demonstrated that NAIs dose-dependently reduce IAV infection of MDCK and A549 cells in the early stage of viral life cycle, in the first hour of viral infection [31]. In addition, direct microscopic observations have been published showing that zanamivir stops the lateral rolling of the viral particles, thereby blocking their movement to the endocytosis-active sites of the plasma membrane [32]. Thus, NAIs are effective blockers of the earliest stages of the IAV life cycle and with timely early admission, they are able to significantly diminish infection of the epithelium of the respiratory tract with a virus.

HA, as the receptor-binding IAV protein, is an attractive molecular target for the pharmacological inhibition and is being actively investigated in this regard. There are two functional activities of the hemagglutinin that are potentially promising for inhibition. The first one is a HA1 subunit-based host cell receptor binding, while the second one is a HA2 subunit-mediated membrane fusion, which is dependent on endosomal low pH. Typically, membrane fusion blockers bind to the HA2 subunit to stabilize HA structure at low pH of the endosome and thereby prevent HA conformational change required for the exposure of the fusion peptide and the performance of membrane fusion. This group of compounds includes such molecules as arbidol (approved for use in Russia and China), Tert-butyl hydroquinone (TBHQ), JNJ4796, CBS1116, positively charged fusion peptide analogues (pFPs), human monoclonal antibodies VIS410 and CR6261, and some others, reviewed elsewhere [93,94].

Another group of HA inhibitors prevents HA1 subunit-dependent reception of SIAs and IAV adsorption. This group includes a number of compounds. Among them, there are several natural substances, such as epicallocatechin-3 gallate (EGCG) from green tea [95], curcumin [96], *Isatis indigotica* root crude extract [97]. These natural substances were shown to effectively reduce viral infection at the earliest stages. In addition, they prevent the reaction of erythrocyte agglutination. These data lead to the conclusion that the binding of HA to sialoglycans of the host cell surface is blocked by these compounds. Further molecular details of the interactions of these natural compounds with hemagglutinin have not yet been clarified.

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Some small molecules are able to effectively block the binding of the IAV to sialic acids of the cellular surface. Of them, oleanolic acid, which is a pentacyclic triterpenoid, effectively inhibits replication of a broad spectrum of IAV subtypes at an early point in the replication cycle [98], as was shown by time-of-addition assay. Effective inhibition of erythrocyte agglutination by oleanolic acid has proved that HA is a molecular target for it. With the help of surface plasmon resonance (SPR) assay, the authors further confirmed the fact of oleanolic acid binding to hemagglutinin. In addition, they directly showed by SPR the blocking of SIA-HA interaction by the oleanolic acid in a dose-dependent manner. Molecular docking also suggested that the SIA- binding receptor pocket within HA is the domain targeted by oleanolic acid [98].

Similarly, prenylated indole diketopiperazine alkaloid Neoechinulin B proved to be a powerful inhibitor of viral infection at the earliest stages [99]. In addition to standard tests such as hemagglutination inhibition, plaque formation assay, time-of-addition experiment, authors of this work also performed a SPR assay to directly demonstrate the dose-dependant inhibition of the SIA-HA binding by the Neoechinulin B.

According to Sacramento et al., another small molecule aureonitol, a tetrahydrofuran derivative, has found similar virus-inhibiting properties [100]. Aureonitol inhibits IAV-based hemagglutination and effectively blocks virus adsorption on the host cell's membrane. Molecular docking revealed that aureonitol enters in the SIA-binding site of hemagglutinin, forming hydrogen bonds with highly conserved residues.

In addition, hemagglutinin-binding DNA- and RNA- aptamers which passed through several rounds of selection show effective inhibition of the hemagglutination reaction, with prospects for their further therapeutic or diagnostic use in influenza treatment [101–103].

Peptides are another group of substances being studied as possible inhibitors of IAV attachment. Jones and co-authors reported that 20-amino-acid peptide EB binds to hemagglutinin and suppresses viral infection of several subtypes in vitro [104]. Using a FITC-labeled virus, authors demonstrated directly that this peptide blocks the adsorption of viral particles on the cell surface. In addition, EB peptide effectively protects mice from H5N1 infection [104]. Later, a similar array of results was obtained for another 12 aa long peptide, called FluPep, or Tkip [105]. The authors reported that FluPep and some related peptides effectively suppress the replication of several subtypes of IAV (H1N1, H3N2 and H5N1), bind to hemagglutinin and prevent the adsorption of the virus on the cell surface. Also, these peptides were able to protect mice from lethal H1N1 infection.

Several neutralizing monoclonal antibodies, such as CH65 [106], C05 [107], S139/1 [108,109] were found to provide cross-reactivity with multiple IAV subtypes. Crystal structures of these antibodies in complex with HA, as well as escape mutant analysis, revealed highly conserved epitopes in HA receptor binding pocket and its surrounding sites. In vivo experiments demonstrated that passive immunization of mice with mAb S139/1 provides effective heterosubtypic protection against H1 and H3 viruses [108].

Information on pharmacological inhibitors of viral protein factors of adsorption is summarized in Table 2.

Table 2. Pharmacological inhibitors of viral factors of IAV adsorption.

Inhibitor	Type of molecule	Viral target	References
Zanamivir	Small molecule	NA, catalytic center	32, 92
Oseltamivir	Small molecule, prodrug	NA, catalytic center	32, 92
Laninamivir	Small molecule, prodrug	NA, catalytic center	92

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Peramivir	Small molecule	NA, catalytic center	92
Epicallocatechin-3 gallate (EGCG)	Natural substance from green tea	HA, H1 subunit	Epicallocatechin-3 gallate (EGCG)
Curcumin	Natural substance	HA, H1 subunit	96
Isatis indigotica root crude extract	Natural substance	HA, H1 subunit	97
Oleanolic acid	Small molecule, tentacyclic triterpenoid	HA, H1 subunit	98
Neoechinulin B	Small molecule, prenylated indole diketopiperazine alkaloid	HA, H1 subunit	99
Aureonitol	Small molecule, tetrahydrofuran derivative	HA, H1 subunit	100
D-12, D-26	RNA-aptamers	HA, H1 subunit	101
Aptamer 1	DNA-aptamer	HA, H1 subunit	102
UHA-2	DNA-aptamer	HA, H1 subunit	103
EB	Peptide	HA, H1 subunit	104
FluPep	Peptide	HA, H1 subunit	105
CH65	Monoclonal antibody	HA, H1 subunit	106
C05	Monoclonal antibody	HA, H1 subunit	107
S139/1	Monoclonal antibody	HA, H1 subunit	108, 109

Thus, the receptor-binding pocket of hemagglutinin and conservative epitopes close to it are promising targets for blocking the adsorption of the virus by various compounds, including small molecules, peptides and antibodies. The search for new inhibitors is actively continuing, in particular on the basis of high-throughput screening protocols for large libraries of small molecules [110].

4.2. *Pharmacological Inhibition of Host Cell Targets of IAV Adsorption.*

In general, cellular targets for the development of new antiviral medicines are favorable because they significantly reduce the problem of emergence of viral resistance and provide the broad-spectrum protection, compared to traditional viral targets. In addition, when inhibiting cellular targets to suppress the IAV infection, in some cases it is possible to re-purpose already known small molecules previously approved for the treatment of other diseases [111,112]. With that, pharmacological manipulation by cellular factors requires especial precaution in terms of safety and possible side effects, given the obvious fact that these molecules have normal functions outside of viral pathogenesis. Despite extensive research, no molecules aimed at cellular targets have been approved and implemented in the clinical practice of influenza treatment to date.

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The most evident cellular target for inhibiting of IAV adsorption is sialic acid, as the main receptor moiety for virus attachment. For this purpose, the DAS181 protein, also known as Fludase, has been developed as a SIA-directed inhibitor of the virus adsorption. DAS181 is a fusion protein composed of a sialidase catalytic domain derived from *Actinomyces viscosus* fused with a cell surface-anchoring sequence [113]. This recombinant sialidase was demonstrated to effectively cleave both 2-6-linked and 2-3-linked SIAs from the cell surface, thereby providing protection against a wide range of IAVs, both in vitro and in vivo [113,114]. DAS181 has successfully passed phase 2 of clinical trials in US [115].

Another compound that has been shown to bind to the host cell's SIAs is a synthetic peptide SALP PEP 19-2.5. Hoffman and colleagues showed that SALP PEP 19-2.5 has low cytotoxicity. Being administered to epithelial cells, this peptide binds to SIAs with high affinity and thus prevents in vitro and in vivo infection by a number of IAV subtypes, including protection against lethal H7N7 infection in mice [116].

As discussed above, the influenza virus uses a number of host cell membrane proteins for adsorption, with following multiple signal transduction events in the area of its attachment. These intense protein interactions launched by IAV attachment are localized in lipid rafts, specialized cholesterol-enriched microdomains of the membrane [63,117]. In this regard, lipid rafts are of interest as a potential target for antiviral agents. The raft-disrupting agent Methyl-β-Cyclodextrin, widely used in research, revealed significant reduction in IAV binding to host cells [117]. In addition, several statins (atorvastatin, fluvastatin) approved for use as cholesterol level-normalizing substances clearly have a protective effect in IAV infection [118]. Similarly, U18666A, an amphipathic cationic amine which inhibits cholesterol biosynthesis and its intracellular transport, reduces IAV entry in a dose-dependent manner [119].

Protein receptors or co-receptors which mediate viral adsorption and internalization, themselves can be considered as promising targets for pharmacological inhibition. In that way, Fujioka and co-authors identified the voltage-dependent Ca^{2+} channel $Ca_v1.2$ as a receptor for IAV's hemagglutinin binding [69]. In that study, the authors successfully used a calcium channel blocker diltiazem to suppress the virus entry into the host cell. Diltiazem is widely assigned in cardiology to reduce hypertension and cardiac arrhythmias, which allows considering the fundamental possibility of its re-purposing as an antiviral drug.

Similarly, EGFR was reported to be an IAV attachment and entry receptor [63], and gefitinib treatment was shown to successfully inhibit the infection in mice [67]. Gefitinib and several new generations of EGFR tyrosine kinase activity inhibitors are approved in oncology, and also could be examined for re-purposing as antiviral drugs.

In addition, there are some laboratory use inhibitors of IAV receptor/co-receptor proteins that were shown to dramatically reduce infection. Such effective inhibition of viral entry was shown for ferristatin II, TfR1 blocker [76]. Likewise, 4-CMTB and compound 58 (Cmp58), which are FFAR2-binding molecules, diminish IAV infection at early time points [78].

At all stages of the life cycle, IAV actively uses numerous protein kinases of the host cell as regulatory and signal-transmitting elements [120,121]. As discussed above, downstream signal transduction in the IAV attachment site also involves several protein kinase signaling pathways. These kinases are being actively investigated as potential targets for pharmacological inhibition in order to stop the virus penetration. Of them, PI3K is activated shortly after attachment of the virus. Recent studies have shown that PI3K inhibitors are able to effectively block viral infection at the stage of IAV entry into the host cell. These PI3K inhibitors include such molecules as wortmannin, LY294002 [122], PIK-75 [112], M85 [123] and Pictilisib, an approved anti-cancer drug [124].

Akt kinase is a downstream component of the PI3K/Akt signaling pathway. Accordingly, pharmacological inhibition of Akt also dramatically reduces IAV infection in the entry stage. Pronounced antiviral effect was demonstrated for such Akt inhibitors as TCL-1 peptide, named "Akt-in" [125], and small molecule inhibitor MK2206 [126].

Focal adhesion kinase (FAK) pathway is also an important component of signaling in the attachment site of the virus. FAK regulates the reorganization of the actin cytoskeleton during IAV

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infection. Bergmann and Elbahesh demonstrated that in case of lethal infection of mice with a H1N1 virus, treatment with a FAK inhibitor Y15 significantly reduces viral load and increases survival [127].

As discussed above, p38MAPK is a downstream signal-transmitting kinase to be activated by TLR4 binding to the IAV. p38MAPK can be inhibited by a small molecule SB203580, with significant decrease in viral entry into infected cells [41].

PLC-γ1 is one more enzyme playing a key role in IAV post-binding signaling. Being activated by RTKs or GPCRs, PLC-γ1 cleaves the phosphatidyl inositol 4,5-bisphosphate (PIP2) into two active molecules, diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP3), both being tightly involved into further regulation of IAV life cycle. It was revealed by Zhu and co-authors that treatment of A549 cells with PLC-γ1 inhibitor U73122 effectively blocks H1N1 virus entry [65].

Data on inhibitors of cellular protein factors of IAV entry are summarized in Table 3.

Table 3. Pharmacological inhibitors of host cell factors of IAV adsorption.

Inhibitor	Type of molecule	Viral target	References
DAS181 protein / Fludase	Recombinant fusion protein	SIA	113, 115
SALP PEP 19-2.5	Peptide	SIA	116
Methyl-β-Cyclodextrin	Small molecule	Lipid rafts	117
Atorvastatin	Small molecule	Lipid rafts	118
Fluvastatin	Small molecule	Lipid rafts	118
U18666A	Small molecule	Lipid rafts	119
Diltiazem	Small molecule	Calcium channels	69
Gefitinib	Small molecule	EGFR	67
Ferristatin II	Small molecule	TfR1	76
4-CMTB	Small molecule	FFAR2	78
Compound 58 (Cmp58)	Small molecule	FFAR2	78
Wortmannin	Small molecule	PI3K	122
LY294002	Small molecule	PI3K	122
PIK-75	Small molecule	PI3K	112
M85	Small molecule	PI3K	123
Pictilisib	Small molecule	PI3K	124
TCL-1 / “Akt-in”	Peptide	Akt	125
MK2206	Small molecule	Akt	126
Y15	Small molecule	FAK	127
SB203580	Small molecule	p38MAPK	41
U73122	Small molecule	PLC-γ1	65

In addition to the above-listed, there are a number of other signaling molecules playing an important roles in the IAV replication cycle, such as PKC, ERK, NF-κB pathway, and many others, also having an extensive set of selective inhibitors The functional activity of these molecules is carried out at later, post-endocytosis stages of the virus life cycle and, for all its obvious importance, is beyond the scope of our review and is discussed elsewhere [120,121].

5. Conclusions

In a number of recent studies considered in our review, a tremendous variety of cellular receptors for binding the influenza A virus has been revealed. These recently identified new hemagglutinin- and neuraminidase- binding proteins have a very different nature, functionally being receptor tyrosine kinases, ion channels, C-lectins, antigen-presenting MHS II molecules, pattern-recognizing receptors, etc. Such a variety of IAV receptors as well as the different signaling cascades associated with them indicates the incredible evolutionary sophistication of this dangerous pathogen.

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Currently, the key issue remains unclear about the possible functional interplay between these multiple receptor mechanisms and signaling pathways in the context of IAV infection. It is unclear, whether they are competitive or mutually complementary when an IAV particle is adsorbed to the host cell.

Plenty of recent data concern the development of new molecules inhibiting IAV adsorption, which would have therapeutic potential for the treatment and prevention of influenza. For future clinical implementation of new candidate compounds, further optimization of their chemical structure is necessary in order to increase their bioavailability and safety. In addition to chemical modification, nanotechnology approaches are prospective, such as encapsulation of active molecules in various nanocontainers, as well as designing 3D scaffolds carrying multivalent active moieties [128,129].

In addition to traditional viral targets for inhibition (NA and HA), researchers pay much attention to cellular factors of virus reception and associated signal transmission as perspective new approaches to the development of drugs to combat influenza. Such new approaches include attempts to therapeutically affect lipid rafts as sites of internalization of the virus, and blocking a number of protein kinases and other enzymes involved in the early stages of infection. In the context of influenza therapy, an attractive cost- and time-saving strategy is to search for regulatory enzyme inhibitors among molecules already approved for the treatment of other diseases, with the perspective of their future re-purposing as antiviral drugs.

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Abbreviations

IAV - Influenza A virus
SIA - N-acetylneuramic (sialic) acid
TEM - transmission electron microscopy
HA - hemagglutinin
NA - neuraminidase
GnT1N - acetylglucosamine transferase 1
CEACAM6 - CEA Cell Adhesion Molecule 6
TLR4 – toll-like receptor 4
PRR - pattern-recognizing receptor
MMR - macrophage mannose receptor
MGL1 - macrophage galactose-type lectin 1
DC – dendritic cell
MHC II - main histocompatibility complex class II
EGFR – epidermal growth factor receptor
CME - clathrin-mediated endocytosis
CIE - clathrin-independent endocytosis
TfR1 - transferrin receptor 1
mGluR2 - metabotropic glutamate receptor subtype 2
FFAR2 - free fatty acid receptor 2
NK - natural killer cell
NAI - Neuraminidase inhibitor
pFPs - positively charged fusion peptide analogues
EGCG - epicallocatechin-3 gallate
SPR - surface plasmon resonance

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FAK - focal adhesion kinase
PLC – phospholipase C
PIP2 - phosphatidyl inositol 4,5-bisphosphate
DAG - diacyl glycerol
IP3 - inositol 1,4,5-trisphosphate
PKC – protein kinase C
ERK - extracellular signal-regulated kinase

References

1. Taubenberger, J.K.; Morens, D.M. Influenza: the once and future pandemic. *Public Health Rep.* **2010** *125, Suppl 3,* 16-26.
2. World Health Organization. *Influenza Fact Sheet.* Available online: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal) (accessed on 05 December 2024).
3. Krammer, F.; Smith, G.J.D.; Fouchier, R.A.M.; Peiris, M,; Kedzierska, K.; Doherty, P.C.; Palese, P.; Shaw, M.L.; Treanor, J.; Webster, R.G.; García-Sastre, A. Influenza. *Nat. Rev. Dis. Primers* **2018,** *4,* 1-21, doi: 10.1038/s41572-018-0002-y.
4. Vasin, A.V.; Temkina, O.A.; Egorov, V.V.; Klotchenko, S.A.; Plotnikova, M.A.; Kiselev, O.I. Molecular mechanisms enhancing the proteome of influenza A viruses: an overview of recently discovered proteins. *Virus Res.* **2014**, *185,* 53-63. doi: 10.1016/j.virusres.2014.03.015.
5. Watanabe, T.; Watanabe, S.; Kawaoka, Y. Cellular networks involved in the influenza virus life cycle. *Cell. Host Microbe* **2010**, *7,* 427-39. doi: 10.1016/j.chom.2010.05.008.
6. König, R.; Stertz, S.; Zhou, Y.; Inoue, A.; Hoffmann, H.H., Bhattacharyya, S.; Alamares, JG.; Tscherne, DM.; Ortigoza, MB.; Liang, Y.; Gao, Q.; Andrews, SE.; Bandyopadhyay, S.; De Jesus, P.; Tu, BP.; Pache, L.; Shih, C.; Orth, A.; Bonamy, G.; Miraglia, L.; Ideker, T.; García-Sastre A.; Young JA.; Palese P.; Shaw M.L.; Chanda S.K. Human host factors required for influenza virus replication. *Nature* **2010,** *463,* 813-817. doi: 10.1038/nature08699.
7. Sempere Borau, M.; Stertz, S. Entry of influenza A virus into host cells - recent progress and remaining challenges. *Curr. Opin. Virol.* **2021,** *48,* 23-29. doi: 10.1016/j.coviro.2021.03.001.
8. Sulpiana.; Amalia R.; Atik N. The Roles of Endocytosis and Autophagy at the Cellular Level During Influenza Virus Infection: A Mini-Review. *Infect. Drug Resist.* **2024.** *17,* 3199-3208. doi: 10.2147/IDR.S471204.
9. Kaksonen, M.; Roux, A. Mechanisms of clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell Biol.* **2018,** *5,* 313-326. doi: 10.1038/nrm.2017.132.
10. Roingeard, P.; Raynal, PI.; Eymieux, S.; Blanchard, E. Virus detection by transmission electron microscopy: Still useful for diagnosis and a plus for biosafety. *Rev. Med. Virol.* **2019,** *1,* e2019. doi: 10.1002/rmv.2019.
11. Lakadamyali, M.; Rust, M.J.; Zhuang, X. Endocytosis of influenza viruses. *Microbes Infect.* **2004**, 10, 929-36. doi: 10.1016/j.micinf.2004.05.002.
12. Sun, E.Z.; Liu, A.A.; Zhang, Z.L.; Liu, S.L.; Tian, Z.Q.; Pang, D.W. Real-time dissection of distinct dynamin-dependent endocytic routes of influenza A virus by quantum dot-based single-virus tracking. *ACS Nano* **2017,** *11,* 4395–4406. https://doi.org/10.1021/acsnano.6b07853.
13. Xie, E..; Ahmad, S..; Smyth, R.P..; Sieben, C. Advanced fluorescence microscopy in respiratory virus cell biology. In *Advances in Virus Research.;* Editor(s): Finke, S.; Ushakov, D.; Academic Press.; *London, United Kingdom,* **2023.**; *Volume 116*.; pp. 123-172. https://doi.org/10.1016/bs.aivir.2023.05.002
14. Zhu, M.; Anirudhan, V.; Du, R.; Rong, L.; Cui, Q. Influenza virus cell entry and targeted antiviral development. *J. Med. Virol.* **2023,** *11,* e29181. doi:10.1002/jmv.29181.
15. Yang, J.; Liu, S. Influenza Virus Entry inhibitors. In: *Virus Entry Inhibitors.; Advances in Experimental Medicine and Biology;* Editor(s): Jiang. S.; Lu L.; Springer, Singapore, **2022,** *Volume* 1366.; pp 123–135. doi: 10.1007/978-981-16-8702-0_8
16. Edinger, T.O.; Pohl, M.O.; Stertz, S. Entry of influenza A virus: host factors and antiviral targets. *J. Gen. Virol.* **2014**, *95(Pt 2),* 263-277. doi: 10.1099/vir.0.059477-0.
17. Liu, WJ.; Xiao, H.; Dai, L.; Liu, D.; Chen, J.; Qi, X.; Bi, Y.; Shi, Y.; Gao, GF.; Liu, Y. Avian influenza A (H7N9) virus: from low pathogenic to highly pathogenic. *Front. Med.* **2021**, *4*, 507-527. doi: 10.1007/s11684-020-0814-5.
18. Karakus, U.; Pohl, M.O.; Stertz, S. Breaking the Convention: Sialoglycan Variants, Coreceptors, and Alternative Receptors for Influenza A Virus Entry. *J. Virol.* ***2020,*** *94,* 10.1128/jvi.01357-19. https://doi.org/10.1128/jvi.01357-19
19. Kamiki, H.; Murakami, S.; Nishikaze, T.; Hiono, T.; Igarashi, M.; Furuse, Y.; Matsugo, H.; Ishida, H.; Katayama, M.; Sekine, W.; Muraki, Y.; Takahashi, M.; Takenaka-Uema, A.; Horimoto, T. Influenza A Virus Agnostic Receptor Tropism Revealed Using a Novel Biological System with Terminal Sialic Acid Knockout Cells. *J. Virol.* **2022,** *96,* e00416-22. https://doi.org/10.1128/jvi.00416-22

20. Lazniewski, M..; Dawson, W.K..; Szczepinska, T.; Plewczynski, D. The structural variability of the influenza A hemagglutinin receptor-binding site. *Brief Funct. Genomics* **2018,** *17,* 415-427.
21. Matrosovich, M.N.; Gambaryan, A.S.; Teneberg, S.; Piskarev, V.E.; Yamnikova, S.S.; Lvov, D.K.; Robertson, J.S.; Karlsson, K.A. 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,* 224-34. doi: 10.1006/viro.1997.8580.
22. de Graaf, M.; Fouchier, R.A. Role of receptor binding specificity in influenza A virus transmission and pathogenesis. *EMBO J.* **2014,** *33,* 823–841. doi: 10.1002/embj.201387442.
23. Guo. X.; Zhou. Y.; Yan. H.; An. Q.; Liang. C.; Liu. L.; Qian. J. Molecular Markers and Mechanisms of Influenza A Virus Cross-Species Transmission and New Host Adaptation. *Viruses* **2024,** *16,* 883. doi: 10.3390/v16060883.
24. Matrosovich, M.; Tuzikov, A.; Bovin, N.; Gambaryan, A.; Klimov, A.; Castrucci, MR.; Donatelli, I.; Kawaoka, Y. Early alterations of the receptor-binding properties of H1.; H2.; and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J. Virol.* **2000,** *18,* 8502-8512. doi: 10.1128/jvi.74.18.8502-8512.2000.
25. Thompson, A.J.; Paulson, J.C. Adaptation of influenza viruses to human airway receptors. *J. Biol. Chem.* **2021,** *296,* 100017. doi: 10.1074/jbc.REV120.013309.
26. Chandrasekaran, A.; Srinivasan, A.; Raman, R.; Viswanathan, K.; Raguram, S.; Tumpey, T.M.; Sasisekharan, V.; Sasisekharan, R. Glycan topology determines human adaptation of avian H5N1 virus hemagglutinin. *Nat. Biotechnol.* **2008**, *1,* 107-13. doi: 10.1038/nbt1375.
27. Varghese JN.; Colman PM. Three-dimensional structure of the neuraminidase of influenza virus A/Tokyo/3/67 at 2.2 A resolution. *J. Mol. Biol.* **1991,** *221,* 473-86. doi: 10.1016/0022-2836(91)80068-6.
28. Shtyrya, Y.A.; Mochalova, L.V.; Bovin, N.V. Influenza virus neuraminidase: structure and function. *Acta Naturae* **2009,** *2*, 26-32.
29. Cohen, M.; Zhang, X.Q.; Senaati, H.P.; Chen, H.W.; Varki, N.M.; Schooley, R.T.; Gagneux, P. Influenza A penetrates host mucus by cleaving sialic acids with neuraminidase. *Virol. J.* **2013,** *10,* 321. doi: 10.1186/1743-422X-10-321.
30. Matrosovich, M.N., Matrosovich, T.Y., Gray, T., Roberts, N.A., Klenk, H.D. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. *J. Virol.* **2004,** *22*, 12665-12667. doi: 10.1128/JVI.78.22.12665-12667.2004.
31. Ohuchi, M.; Asaoka, N.; Sakai, T.; Ohuchi, R. Roles of neuraminidase in the initial stage of influenza virus infection. *Microbes Infect.* **2006,** *5,* 1287-1293. doi: 10.1016/j.micinf.2005.12.008.
32. Sakai, T.; Nishimura, S.I.; Naito, T.; Saito, M. Influenza A virus hemagglutinin and neuraminidase act as novel motile machinery. *Sci Rep.* **2017**, *7*, 45043. doi: 10.1038/srep45043.
33. Guo, H.; Rabouw,H.; Slomp, A.; Dai, M.; van der Vegt, F.; van Lent, J.W.M.; McBride, R.; Paulson, J.C.; de Groot, R.J.; van Kuppeveld, F.J.M.; de Vries, E.; de Haan, C.A.M. Kinetic analysis of the influenza A virus HA/NA balance reveals contribution of NA to virus-receptor binding and NA-dependent rolling on receptor-containing surfaces. *PLoS Pathog.* **2018.** *14*, e1007233. doi: 10.1371/journal.ppat.1007233.
34. de Vries, E.; Du, W.; Guo, H.; de Haan, C.A.M. Influenza A Virus Hemagglutinin-Neuraminidase-Receptor Balance: Preserving Virus Motility. *Trends Microbiol.* **2020**, *1*, 57-67. doi: 10.1016/j.tim.2019.08.010.
35. Gaymard, A.; Le Briand, N.; Frobert, E.; Lina, B.; Escuret, V. Functional balance between neuraminidase and haemagglutinin in influenza viruses. *Clin Microbiol Infect.* **2016,** *12,* 975-983. doi: 10.1016/j.cmi.2016.07.007.
36. de Vries, E.; de Vries, R.P.; Wienholts, M.J.; Floris, C.E.; Jacobs, M.S.; van den Heuvel, A.; Rottier P.J.; de Haan, C.A. Influenza A virus entry into cells lacking sialylated N-glycans. *Proc. Natl. Acad. Sci. USA* **2012**, *19*, 7457-62. doi: 10.1073/pnas.1200987109.
37. Stray, S.J.; Cummings, R.D.; Air, G.M. Influenza virus infection of desialylated cells. *Glycobiology* **2000**, *7*, 649-58. doi: 10.1093/glycob/10.7.649.
38. Thompson, C. I.; Barclay, W. S.; Zambon, M. C.; Pickles, R. J. Infection of human airway epithelium by human and avian strains of influenza a virus. *J. Virol.* **2006,** *80,* 8060–8068. doi: 10.1128/JVI.00384-06.
39. Byrd-Leotis, L.; Jia, N.; Dutta, S.; Trost, JF.; Gao, C.; Cummings, S.F.; Braulke, T.; Müller-Loennies, S.; Heimburg-Molinaro, J.; Steinhauer, D.A.; Cummings, R.D. Influenza binds phosphorylated glycans from human lung. *Sci. Adv.* **2019.** *5,* eaav2554. doi: 10.1126/sciadv.aav2554.
40. Rahman, S.K; Ansari, M.A; Gaur, P.; Ahmad. I.; Chakravarty, C.; Verma, D.K.; Sharma, A.; Chhibber, S.; Nehal, N.; Wirth, D.; Lal, S.K. The Immunomodulatory CEA Cell Adhesion Molecule 6 (CEACAM6/CD66c) Is a Protein Receptor for the Influenza a Virus. *Viruses* **2021**, *5,* 726. doi: 10.3390/v13050726.
41. Marchant, D.; Singhera, G.K.; Utokaparch, S.; Hackett, T.L.; Boyd, J.H.; Luo, Z.; Si, X.; Dorscheid, D.R.; McManus, B.M.; Hegele, R.G. Toll-like receptor 4-mediated activation of p38 mitogen-activated protein kinase is a determinant of respiratory virus entry and tropism. *J. Virol.* **2010**, *84*, 11359–11373. https://doi.org/10.1128/JVI.00804-10

42. Reading, P.C.; Miller, J.L.; Anders, E.M. Involvement of the mannose receptor in infection of macrophages by influenza virus. *J. Virol.* **2000,** *11,* 5190-7. doi: 10.1128/jvi.74.11.5190-5197.2000.
43. Upham, J.P.; Pickett, D.; Irimura, T.; Anders, E.M.; Reading, P.C. Macrophage receptors for influenza A virus: role of the macrophage galactose-type lectin and mannose receptor in viral entry. *J. Virol.* **2010,** *8,* 3730-3737. doi: 10.1128/JVI.02148-09.
44. Ng, W.C.; Liong, S.; Tate, M.D.; Irimura, T.; Denda-Nagai, K.; Brooks, A.G.; Londrigan, S.L.; Reading, P.C. The macrophage galactose-type lectin can function as an attachment and entry receptor for influenza virus. *J. Virol.* **2014,** *3,* 1659-1672. doi: 10.1128/JVI.02014-13.
45. Ng, W.C.; Londrigan, S.L.; Nasr, N.; Cunningham, A.L.; Turville, S.; Brooks, A.G.; Reading, P.C. The C-type Lectin Langerin Functions as a Receptor for Attachment and Infectious Entry of Influenza A Virus. *J. Virol.* **2015**, *1,* 206-221. doi: 10.1128/JVI.01447-15.
46. Wang, S.F.; Huang, J.C.; Lee, Y.M.; Liu, S.J.; Chan, Y.J.; Chau, Y.P.; Chong, P.; Chen, Y.M. DC-SIGN mediates avian H5N1 influenza virus infection in cis and in trans. *Biochem. Biophys. Res. Commun.* **2008,** *4,* 561-566. doi: 10.1016/j.bbrc.2008.06.078.
47. Londrigan, S.L.; Turville, S.G.; Tate, M.D.; Deng, Y.M.; Brooks, A.G.; Reading, P.C. 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,** *6,* 2990-3000. doi: 10.1128/JVI.01705-10.
48. Hillaire, M.L.; Nieuwkoop, N.J.; Boon, A.C.; de Mutsert, G.; Vogelzang-van Trierum, S.E.; Fouchier, R.A.; Osterhaus, A.D.; Rimmelzwaan, G.F. Binding of DC-SIGN to the hemagglutinin of influenza A viruses supports virus replication in DC-SIGN expressing cells. *PLoS One* **2013**, *8*, e56164. doi: 10.1371/journal.pone.0056164.
49. Gillespie, L.; Roosendahl, P.; Ng, WC.; Brooks, A.G.; Reading, P.C.; Londrigan, S.L. Endocytic function is critical for influenza A virus infection via DC-SIGN and L-SIGN. *Sci. Rep.* **2016,** *6,* 19428. doi: 10.1038/srep19428.
50. Rodgers, B.; Mims, C.A. Interaction of influenza virus with mouse macrophages. *Infect. Immun.* **1981**, *2*, 751-757. doi: 10.1128/iai.31.2.751-757.1981.
51. Reading, P.C.; Whitney, P.G.; Pickett, D.L.; Tate, M.D.; Brooks, A.G. Influenza viruses differ in ability to infect macrophages and to induce a local inflammatory response following intraperitoneal injection of mice. *Immunol. Cell Biol.* **2010,** *88,* 641–650. http://dx.doi.org/10.1038/icb.2010.11.
52. Ioannidis, L.J.; Verity, E.E.; Crawford, S.; Rockman, S.P.; Brown, L.E. Abortive replication of influenza virus in mouse dendritic cells. *J. Virol.* **2012,** *86,* 5922-5925. doi: 10.1128/JVI.07060-11.
53. Tate, M.D.; Schilter, H.C.; Brooks, A.G.; Reading, P.C. Responses of mouse airway epithelial cells and alveolar macrophages to virulent and avirulent strains of influenza A virus. *Viral Immunol.* **2011**, *24,* 77–88. http://dx .doi.org/10.1089/vim.2010.0118.
54. Palomino-Segura, M.; Perez, L.; Farsakoglu, Y.; Virgilio, T.; Latino, I.; D'Antuono, R.; Chatziandreou, N.; Pizzagalli, D.U.; Wang, G.; García-Sastre, A.; Sallusto, F.; Carroll, M.C.; Neyrolles, O.; Gonzalez, S.F. Protection against influenza infection requires early recognition by inflammatory dendritic cells through C-type lectin receptor SIGN-R1. *Nat. Microbiol.* **2019**, *4,* 1930-1940. doi: 10.1038/s41564-019-0506-6.
55. Sun, X.; Shi, Y.; Lu, X.; He, J.; Gao, F.; Yan, J.; Qi, J.; Gao G.F. Bat-derived influenza hemagglutinin H17 does not bind canonical avian or human receptors and most likely uses a unique entry mechanism. *Cell Rep.* **2013**, *3,* 769-78. doi: 10.1016/j.celrep.2013.01.025.
56. Tong, S.; Zhu, X.; Li, Y.; Shi, M.; Zhang, J.; Bourgeois, M.; Yang, H.; Chen ,X.; Recuenco, S.; Gomez, J.; Chen, L.M.; Johnson, A.; Tao, Y.; Dreyfu,s C.; Yu, W.; McBride, R.; Carney, P.J.; Gilbert, A.T.; Chang, J.; Guo, Z.; Davis, C.T.; Paulson, J.C.; Stevens, J.; Rupprecht, C.E.; Holmes, E.C.; Wilson, I.A.; Donis, R.O. New world bats harbor diverse influenza A viruses. *PLoS Pathog.* **2013**, *9*, e1003657. doi: 10.1371/journal.ppat.1003657.
57. Karakus, U.; Thamamongood, T.; Ciminski, K.; Ran, W.; Günther, S.C.; Pohl, M.O.; Eletto, D.; Jeney, C.; Hoffmann, D.; Reiche, S.; Schinköthe, J.; Ulrich, R.; Wiener, J.; Hayes, M.G.B.; Chang, M.W.; Hunziker, A.; Yángüez, E.; Aydillo, T.; Krammer, F.; Oderbolz, J.; Meier, M.; Oxenius, A.; Halenius, A.; Zimmer, G.; Benner, C.; Hale, B.G.; García-Sastre, A.; Beer, M.; Schwemmle, M.; Stertz, S. MHC class II proteins mediate cross-species entry of bat influenza viruses. *Nature* **2019,** *567,* 109-112. doi: 10.1038/s41586-019-0955-3.
58. Karakus, U.; Sempere Borau, M.; Martínez-Barragán, P.; von Kempis, J.; Yildiz S.; Arroyo-Fernández, L.M.; Pohl, M.O.; Steiger, J.A.; Glas, I.; Hunziker, A.; García-Sastre, A.; Stertz, S. MHC class II proteins mediate sialic acid independent entry of human and avian H2N2 influenza A viruses. *Nat. Microbiol.* **2024,** *9,* 2626-2641. doi: 10.1038/s41564-024-01771-1.
59. Dadonaite, B.; Vijayakrishnan, S.; Fodor, E.; Bhella, D.; Hutchinson, E.C. Filamentous influenza viruses. *J. Gen. Virol.* **2016**, *97*, 1755-1764. doi: 10.1099/jgv.0.000535.
60. Badham, M.D.; Rossman, J.S. Filamentous Influenza Viruses. *Curr. Clin. Microbiol. Rep.* **2016**, *3,* 155-161. doi: 10.1007/s40588-016-0041-7.
61. Li, T.; Li, Z.; Deans, E.E.; Mittler, E.; Liu, M.; Chandran, K.; Ivanovic, T. The shape of pleomorphic virions determines resistance to cell-entry pressure. *Nat. Microbiol.* **2021,** *6,* 617-629. doi: 10.1038/s41564-021-00877-0.

62. Weis, W.; Brown, J.H.; Cusack, S.; Paulson, J.C.; Skehel, J.J.; Wiley, D.C. Structure of the influenza virus haemagglutinin complexed with its receptor, sialic acid. *Nature* **1988,** *333,* 426-431. doi: 10.1038/333426a0.
63. Eierhoff, T.; Hrincius, E.R.; Rescher, U.; Ludwig, S.; Ehrhardt, C. The epidermal growth factor receptor (EGFR) promotes uptake of influenza A viruses (IAV) into host cells. *PLoS Pathog.* **2010**, *6*, e1001099. doi: 10.1371/journal.ppat.1001099.
64. Elbahesh, H.; Cline, T.; Baranovich, T.; Govorkova, E.A.; Schultz-Cherry, S.; Russell, C.J. Novel roles of focal adhesion kinase in cytoplasmic entry and replication of influenza A viruses. J. Virol. 2014, 88, 6714-6728. doi: 10.1128/JVI.00530-14.
65. Zhu, L.; Ly, H.; Liang, Y. PLC-γ1 signaling plays a subtype-specific role in postbinding cell entry of influenza A virus. *J. Virol.* **2014**, *88*, 417-424. doi: 10.1128/JVI.02591-13.
66. O'Hanlon, R.; Leyva-Grado, V.H.; Sourisseau, M.; Evans, M.J.; Shaw, M.L. An influenza virus entry inhibitor targets class II PI3 kinase and synergizes with oseltamivir. *ACS Infect. Dis.* **2019,** *5,* 1779 –1793. /doi.org/10.1021/ acsinfecdis.9b00230.
67. Mitchell, H.D.; Eisfeld, A.J.; Stratton, K.G.; Heller, N.C.; Bramer, L.M.; Wen, J.; McDermott, J.E.; Gralinski, L.E.; Sims, A.C.; Le, M.Q.; Baric, R.S.; Kawaoka, Y.; Waters K.M. The Role of EGFR in Influenza Pathogenicity: Multiple Network-Based Approaches to Identify a Key Regulator of Non-lethal Infections. *Front. Cell. Dev. Biol.* **2019**, *7*, 200. doi: 10.3389/fcell.2019.00200.
68. Sieben, C.; Sezgin, E.; Eggeling, C.; Manley, S. Influenza A viruses use multivalent sialic acid clusters for cell binding and receptor activation. *PLoS Pathog.* **2020**, *16*, e1008656. doi: 10.1371/journal.ppat.1008656.
69. Fujioka, Y.; Nishide, S.; Ose ,T.; Suzuki, T.; Kato I.; Fukuhara H.; Fujioka M.; Horiuchi K.; Satoh AO.; Nepal P.; Kashiwagi S.; Wang J.; Horiguchi M.; Sato Y.; Paudel S.; Nanbo A.; Miyazaki T.; Hasegawa H.; Maenaka K.; Ohba Y. A Sialylated Voltage-Dependent Ca^{2+} Channel Binds Hemagglutinin and Mediates Influenza A Virus Entry into Mammalian Cells. *Cell Host Microbe* **2018,** *23,* 809-818.e5. doi: 10.1016/j.chom.2018.04.015.
70. Bao, M.-N..; Zhang, L.-J.; Tang, B.; Fu, D.-D; Li, J.; Du, L.; Hou, Y.-N.; Zhang, Z.-L.; Tang, H.-W.; Pang, D.-W. Influenza A Viruses Enter Host Cells via Extracellular Ca^{2+} Influx-Involved Clathrin-Mediated Endocytosis. *ACS Applied Bio Materials* **2021,** *4,* 2044-2051. DOI: 10.1021/acsabm.0c00968.
71. Fujioka, Y.; Tsuda, M.; Nanbo, A.; Hattori, T.; Sasaki, J.; Sasaki, T.; Miyazaki, T.; Ohba, Y. A Ca(2+)-dependent signalling circuit regulates influenza A virus internalization and infection. *Nat. Commun.* **2013,** *4,* 2763. doi: 10.1038/ncomms3763.
72. Yan, Y.; Du, Y.; Wang, G.; Li, K. Non-structural protein 1 of H3N2 influenza A virus induces nucleolar stress via interaction with nucleolin. *Sci. Rep.* ***2017,*** *7,* 17761. doi: 10.1038/s41598-017-18087-2.
73. Terrier, O.; Carron, C.; De Chassey, B.; Dubois, J.; Traversier, A.; Julien, T.; Cartet, G.; Proust, A.; Hacot, S.; Ressnikoff, D.; Lotteau, V.; Lina, B.; Diaz, J.J.; Moules, V.; Rosa-Calatrava M. Nucleolin interacts with influenza A nucleoprotein and contributes to viral ribonucleoprotein complexes nuclear trafficking and efficient influenza viral replication. *Sci. Rep.* **2016**, *4,* 29006. doi: 10.1038/srep29006.
74. Gao, Z.; Hu, J.; Wang, X.; Yang, Q.; Liang, Y.; Ma, C.; Liu, D.; Liu, K.; Hao, X.; Gu, M.; Liu X.; Jiao, X.A.; Liu, X. The PA-interacting host protein nucleolin acts as an antiviral factor during highly pathogenic H5N1 avian influenza virus infection. *Arch. Virol.* **2018,** *163,* 2775-2786. doi: 10.1007/s00705-018-3926-3.
75. Chan, C.M.; Chu, H.; Zhang, A.J.; Leung, L.H.; Sze, K.H.; Kao, R.Y.; Chik, K.K.; To, K.K.; Chan, J.F.; Chen H.; Jin, D,Y.; Liu, L.; Yuen KY. Hemagglutinin of influenza A virus binds specifically to cell surface nucleolin and plays a role in virus internalization. Virology 2016, 494, 78-88. doi: 10.1016/j.virol.2016.04.008.
76. Mazel-Sanchez, B.; Niu, C.; Williams, N.; Bachmann, M.; Choltus, H.; Silva, F.; Serre-Beinier, V.; Karenovics, W.; Iwaszkiewicz, J.; Zoete, V.; Kaiser, L.; Hartley, O.; Wehrle-Haller, B.; Schmolke M. Influenza A virus exploits transferrin receptor recycling to enter host cells. *Proc. Natl. Acad. Sci. U S A. 2023, 120,* e2214936120. doi: 10.1073/pnas.2214936120.
77. Ni, Z.; Wang, J.; Yu, X.; Wang, Y.; Wang, J.; He, X.; Li, C.; Deng, G.; Shi, J.; Kong, H.; Jiang, Y.; Chen, P.; Zeng, X.; Tian, G.; Chen, H.; Bu, Z. Influenza virus uses mGluR2 as an endocytic receptor to enter cells. *Nat. Microbiol.* **2024,** *9,* 1764-1777. doi: 10.1038/s41564-024-01713-x.
78. Wang, G.; Jiang, L.; Wang, J.; Zhang, J.; Kong, F.; Li, Q.; Yan, Y.; Huang, S.; Zhao Y.; Liang L.; Li J.; Sun N.; Hu Y.; Shi W.; Deng G.; Chen P.; Liu L.; Zeng X.; Tian G.; Bu Z.; Chen H.; Li C. The G Protein-Coupled Receptor FFAR2 Promotes Internalization during Influenza A Virus Entry. *J. Virol.* **2020,** *94,* e01707-19. doi: 10.1128/JVI.01707-19.
79. Mandelboim, O.; Lieberman, N.; Lev, M.; Paul, L.; Arnon, TI.; Bushkin, Y.; Davis, D.M.; Strominger, J.L.; Yewdell, J.W.; Porgador, A. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* **2001,** *409,* 1055-1060. doi: 10.1038/35059110.
80. Ho, J.W.; Hershkovitz, O.; Peiris, M.; Zilka, A.; Bar-Ilan, A.; Nal, B.; Chu, K.; Kudelko, M.; Kam, Y.W.; Achdout, H.; Mandelboim, M.; Altmeyer, R.; Mandelboim, O.; Bruzzone, R.; Porgador, A. H5-type influenza virus hemagglutinin is functionally recognized by the natural killer-activating receptor NKp44. *J. Virol.* **2008,** *82,* 2028-32. doi: 10.1128/JVI.02065-07.
81. Mendelson, M.; Tekoah, Y.; Zilka ,A.; Gershoni-Yahalom, O.; Gazit, R.; Achdout, H.; Bovin, N.V.; Meningher, T.; Mandelboim, M.; Mandelboim, O.; David, A.; Porgador, A. NKp46 O-glycan sequences that

are involved in the interaction with hemagglutinin type 1 of influenza virus. *J. Virol.* **2010**, *84,* 3789-3797. doi: 10.1128/JVI.01815-09.

82. Arnon, T.I.; Achdout, H.; Lieberman, N.; Gazit, R.; Gonen-Gross, T.; Katz, G.; Bar-Ilan, A.; Bloushtain, N.; Lev, M.; Joseph, A.; Kedar, E.; Porgador, A.; Mandelboim, O. The mechanisms controlling the recognition of tumor- and virus-infected cells by NKp46. *Blood* **2004**, *103,* 664-72. doi: 10.1182/blood-2003-05-1716.
83. Glasner, A.; Zurunic, A.; Meningher, T.; Lenac Rovis, T.; Tsukerman, P.; Bar-On, Y.; Yamin, R.; Meyers, A.F.; Mandeboim, M.; Jonjic, S.; Mandelboim, O. Elucidating the mechanisms of influenza virus recognition by Ncr1. *PLoS One* **2012,** *7,* e36837. doi: 10.1371/journal.pone.0036837.
84. Glasner, A.; Roth, Z.; Varvak, A.; Miletic, A.; Isaacson, B.; Bar-On, Y.; Jonjic, S.; Khalaila, I.; Mandelboim, O. Identification of putative novel O-glycosylations in the NK killer receptor Ncr1 essential for its activity. *Cell Discov.* **2015,** *1, 15036.* doi: 10.1038/celldisc.2015.36.
85. Luczo, J.M.; Ronzulli, S.L.; Tompkins, S.M. Influenza A Virus Hemagglutinin and Other Pathogen Glycoprotein Interactions with NK Cell Natural Cytotoxicity Receptors NKp46, NKp44, and NKp30. *Viruses* 2021, *13, 156*. doi: 10.3390/v13020156.
86. Mao, H.; Tu, W.; Qin, G.; Law, H.K.; Sia, S.F.; Chan, P.L.; Liu, Y.; Lam, K.T.; Zheng, J.; Peiris, M.; Lau, Y.L. Influenza virus directly infects human natural killer cells and induces cell apoptosis. *J. Virol.* 2009, *83,* 9215-9222. doi: 10.1128/JVI.00805-09.
87. Rossman, J.S.; Leser, G.P.; Lamb, R.A. Filamentous influenza virus enters cells via macropinocytosis. *J. Virol.* 2012, *86,* 10950-10960. doi:10.1128/JVI.05992-11 818
88. Chen, C.; Zhuang, X. Epsin 1 is a cargo-specific adaptor for the clathrin-mediated endocytosis of the influenza virus. *Proc. Natl. Acad. Sci. U S A* 2008, *105, 11790-11795.* doi: 10.1073/pnas.0803711105.
89. Perwitasari, O.; Yan, X.; O'Donnell, J.; Johnson, S.; Tripp, R.A. Repurposing Kinase Inhibitors as Antiviral Agents to Control Influenza A Virus Replication. *Assay Drug Dev. Technol.* 2015, *13,* 638-649. doi: 10.1089/adt.2015.0003.drrr.
90. Chou, Y.C.; Lai, M.M.; Wu, Y.C.; Hsu, N.C.; Jeng, K.S.; Su, W.C. Variations in genome-wide RNAi screens: lessons from influenza research. *J. Clin. Bioinforma.* **2015**, *5, 2.* doi: 10.1186/s13336-015-0017-5.
91. Maes, A.; Botzki, A.; Mathys, J.; Impens, F.; Saelens, X. Systematic review and meta-analysis of genome-wide pooled CRISPR screens to identify host factors involved in influenza A virus infection. *J. Virol.* **2024,** *98,* e0185723. doi: 10.1128/jvi.01857-23.
92. Wang, J.; Shen, B.; Yue, L.; Xu, H.; Chen, L.; Qian, D.; Dong, W.; Hu, Y. The Global Trend of Drug Resistant Sites in Influenza A Virus Neuraminidase Protein from 2011 to 2020. *Microorganisms* **2024,** *12, 2056.* doi: 10.3390/microorganisms12102056.
93. Zhu, M.; Anirudhan, V.; Du, R.; Rong, L.; Cui, Q. Influenza virus cell entry and targeted antiviral development. *J. Med. Virol.* **2023,** *95,* e29181. doi: 10.1002/jmv.29181.
94. Hermoso-Pinilla, F.J.; Valdivia, A.; Camarasa, M.J.; Ginex, T.; Luque, F.J. Influenza A virus hemagglutinin: from classical fusion inhibitors to proteolysis targeting chimera-based strategies in antiviral drug discovery. *Explor. Drug. Sci.* **2024,** *2,* 85–116. https://doi.org/10.37349/eds.2024.00037
95. Kim, M.; Kim, S.Y.; Lee, H.W.; Shin, J.S.; Kim, P.; Jung, Y.S.; Jeong, H.S.; Hyun, J.K.; Lee, C.K. Inhibition of influenza virus internalization by (-)-epigallocatechin-3-gallate. *Antiviral Res.* **2013,** *100,* 460-472. doi: 10.1016/j.antiviral.2013.08.002.
96. Chen, D.Y.; Shien, J.H.; Tiley, L.; Chiou, Wang, S.-S.; Chang, T.-J.; Lee Y.-J.; Chan, K.-W.; Hsu, W.-L. Curcumin inhibits influenza virus infection and haemagglutination activity. *Food Chem.* **2010,** *119,* 1346-1351. doi:10.1016/j.foodchem.2009.09.011
97. Yang, Z.; Wang, Y.; Zhong, S.; Zhao, S.; Zeng, X.; Mo, Z.; Qin, S.; Guan, W.; Li, C.; Zhong, N. In vitro inhibition of influenza virus infection by a crude extract from Isatis indigotica root resulting in the prevention of viral attachment. *Mol. Med. Rep.* **2012,** *5,* 793-799. doi: 10.3892/mmr.2011.709.
98. Yu, M.; Si, L.; Wang, Y.; Wu, Y.; Yu, F.; Jiao, P.; Shi, Y.; Wang, H.; Xiao, S.; Fu, G.; Tian, K.; Wang, Y.; Guo, Z.; Ye, X.; Zhang, L.; Zhou, D. Discovery of pentacyclic triterpenoids as potential entry inhibitors of influenza viruses. *J. Med. Chem.* **2014,** *57,* 10058-10071. doi: 10.1021/jm5014067.
99. Chen X.; Si L.; Liu D.; Proksch P.; Zhang L.; Zhou D.; Lin W. Neoechinulin B and its analogues as potential entry inhibitors of influenza viruses.; targeting viral hemagglutinin. *Eur. J. Med. Chem.* **2015**, *93,* 182-195. doi: 10.1016/j.ejmech.2015.02.006.
100. Sacramento, C.Q.; Marttorelli, A.; Fintelman-Rodrigues, N.; de Freitas, C.S.; de Melo, G.R.; Rocha, M.E.; Kaiser, C.R.; Rodrigues, K.F.; da Costa, G.L.; Alves, C.M.; Santos-Filho, O.; Barbosa, J.P.; Souza, T.M. Aureonitol.; a Fungi-Derived Tetrahydrofuran.; Inhibits Influenza Replication by Targeting Its Surface Glycoprotein Hemagglutinin. *PLoS ONE* **2015**, *10,* e0139236. doi: 10.1371/journal.pone.0142246.
101. Gopinath, S.C.B.; Kumar, P.K.R. Aptamers that bind to the hemagglutinin of the recent pandemic influenza virus H1N1 and efficiently inhibit agglutination. *Acta Biomater.* **2013,** *9,* 8932-8941. doi:10.1016/j.actbio.2013.06.016
102. Li, W.; Feng ,X.; Yan, X.; Liu, K.; Deng, L. A DNA aptamer against influenza A virus: an effective inhibitor to the hemagglutinin-glycan interactions. *Nucleic Acid Ther.* **2016**, *26,* 166-172. doi:10.1089/nat.2015.0564

103. Wang, M.; Hao, MC.; Huangfu, Y.; Yang, KZ.; Zhang, XQ.; Zhang, Y.; Chen, J.; Zhang, ZL. A Universal Aptamer for Influenza A Viruses: Selection, Recognition, and Infection Inhibition. *ACS Pharmacol. Transl. Sci.* **2023,** *7,* 249-258. doi: 10.1021/acsptsci.3c00258.
104. Jones, J.C.; Turpin, E.A.; Bultmann, H.; Brandt, C.R.; Schultz-Cherry, S. Inhibition of influenza virus infection by a novel antiviral peptide that targets viral attachment to cells. *J. Virol.* **2006,** *80,* 11960-11967. doi: 10.1128/JVI.01678-06.
105. Nicol, M.Q.; Ligertwood, Y.; Bacon, M.N.; Dutia, B.M.; Nash, A.A. A novel family of peptides with potent activity against influenza A viruses. *J. Gen. Virol.* **2012,** *93,* 980-986. doi: 10.1099/vir.0.038679-0.
106. Whittle, J.R.R.; Zhang, R.J.; Khurana, S.; King LR, Manischewitz, J.; Golding, H.; Dormitzer, P.R.; Haynes, B.F.; Walter, E.B.; Moody, M.A.; Kepler, T.B.; Liao, H.X.; Harrison, S.C. Broadly neutralizing human antibody that recognizes the receptor-binding pocket of influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA* **2011,** *108,* 14216-14221. doi:10.1073/pnas.1111497108
107. Ekiert, D.C.; Kashyap, A.K.; Steel, J.; Rubrum, A.; Bhabha, G.; Khayat, R.; Lee, J.H.; Dillon, M.A.; O'Neil, R.E.; Faynboym, A.M.; Horowitz, M.; Horowitz, L.; Ward, A.B.; Palese, P.; Webby, R.; Lerner, R.A.; Bhatt, R.R.; Wilson, I.A. Cross-neutralization of influenza A viruses mediated by a single antibody loop. *Nature* **2012**, *489,* 526-532. doi: 10.1038/nature11414
108. Yoshida R.; Igarashi M.; Ozaki H.; Kishida, N.; Tomabechi, D.; Kida, H.; Ito, K.; Takada, A. Cross-Protective potential of a novel monoclonal antibody directed against antigenic site B of the hemagglutinin of influenza A viruses. *PLoS Pathog.* **2009,** *5,* e1000350. doi:10.1371/journal.ppat.1000350
109. Lee, P.S.; Yoshida, R.; Ekiert, D.C.; Sakai, N.; Suzuki, Y.; Takada, A.; Wilson, I.A. Heterosubtypic antibody recognition of the influenza virus hemagglutinin receptor binding site enhanced by avidity. *Proc. Natl. Acad. Sci. USA* **2012,** *109,* 17040-17045. doi:10.1073/pnas.1212371109
110. Weisshaar, M.; Cox, R.; Morehouse, Z.; Kyasa, S.K.; Yan, D.; Oberacker, P.; Mao S.; Golden, J.E.; Lowenm A,C.; Natchus, M,G.; Plemper, R.K. Identification and characterization of influenza virus entry inhibitors through dual myxovirus high-throughput screening. *J. Virol.* **2016,** *90,* 7368 –7387. doi:10.1128/JVI.00898-16.
111. Terrier, O.; Slama-Schwok, A. Anti-Influenza Drug Discovery and Development: Targeting the Virus and Its Host by All Possible Means. *Adv. Exp. Med. Biol.* **2021**, *1322,* 195-218. doi: 10.1007/978-981-16-0267-2_8.
112. Perwitasari, O.; Yan, X,; O'Donnell, J.; Johnson, S.; Tripp, R.A. Repurposing Kinase Inhibitors as Antiviral Agents to Control Influenza A Virus Replication. *Assay Drug Dev. Technol.* **2015,** *13,* 638-649. doi: 10.1089/adt.2015.0003.drrr.
113. Malakhov, M.P.; Aschenbrenner, L.M.; Smee, D.F.; Wandersee, M.K.; Sidwell, R,W.; Gubarevam L.V.; Mishin, V.P.; Hayden, F.G.; Kim, D.H.; Ing, A.; Campbell, E.R.; Yu, M.; Fang, F. Sialidase fusion protein as a novel broad-spectrum inhibitor of influenza virus infection. *Antimicrob. Agents Chemother.* **2006,** *50,* 1470-1479. doi: 10.1128/AAC.50.4.1470-1479.2006.
114. Belser, J.A.; Lu, X.; Szretter, K.J.; Jin, X.; Aschenbrenner, L.M.; Lee, A.; Hawley, S.; Kim, D.H.; Malakhov, M.P.; Yum, M.; Fang, F.; Katz, J.M. DAS181, a novel sialidase fusion protein, protects mice from lethal avian influenza H5N1 virus infection. *J. Infect. Dis.* **2007,** *196,* 1493-1499. doi: 10.1086/522609.
115. Koszalka, P.; Tilmanis, D.; Hurt, A.C. Influenza antivirals currently in late-phase clinical trial. *Influenza Other Respir Viruses* **2017**, *11,* 240-246. doi: 10.1111/irv.12446.
116. Hoffmann, J.; Schneider, C.; Heinbockel, L.; Brandenburg, K.; Reimer, R.; Gabriel, G. A new class of synthetic anti-lipopolysaccharide peptides inhibits influenza A virus replication by blocking cellular attachment. *Antiviral Res.* **2014,** *104,* 23-33. doi:10.1016/j.antiviral.2014.01.015.
117. Verma, D.K.; Gupta, D.; Lal, S.K. Host Lipid Rafts Play a Major Role in Binding and Endocytosis of Influenza A Virus. *Viruses* **2018,** *10,* 650. doi: 10.3390/v10110650.
118. Li, Y.-J.; Chen, C.-Y.; Yang, J.-H.; Chiu, Y.-F. Modulating cholesterol-rich lipid rafts to disrupt influenza A virus infection. *Front. Immunol.* **2022,** *13,* 982264. doi: 10.3389/fimmu.2022.982264
119. Li, G.; Su, B.; Fu, P.; Bai, Y.; Ding, G.; Li, D.; Wang, J.; Yang, G.; and Chu, B. NPC1-regulated dynamic of clathrin-coated pits is essential for viral entry. *Sci. China Life Sci.* **2022,** *65,* 341–361. https://doi.org/10.1007/s11427-021-1929-y
120. Meineke, R.; Rimmelzwaan, G.F.; Elbahesh, H. Influenza Virus Infections and Cellular Kinases. *Viruses* **2019,** *11, 71.* doi: 10.3390/v11020171.
121. Dey, S.; Mondal, A. Unveiling the role of host kinases at different steps of influenza A virus life cycle. *J. Virol.* **2024,** *98,* e0119223. doi: 10.1128/jvi.01192-23.
122. Hsu, A.C.; Starkey, M.R.; Hanish, I.; Parsons, K.; Haw, T.J.; Howland, LJ.; Barr, I.; Mahony, J.B.; Foster, P.S.; Knight, D.A.; Wark, P.A.; Hansbro, P.M. Targeting PI3K-p110α Suppresses Influenza Virus Infection in Chronic Obstructive Pulmonary Disease. *Am. J. Respir. Crit. Care Med.* **2015,** *191, 1012-1023.* doi: 10.1164/rccm.201501-0188OC.
123. O'Hanlon, R.; Leyva-Grado, VH.; Sourisseau, M.; Evans, M.J.; Shaw, M.L. An Influenza Virus Entry Inhibitor Targets Class II PI3 Kinase and Synergizes with Oseltamivir. *ACS Infect. Dis.* **2019,** *5, 1779-1793.* doi: 10.1021/acsinfecdis.9b00230.

124. Deinhardt-Emmer, S.; Jäckel, L.; Häring, C.; Böttcher, S.; Wilden, J.J.; Glück, B.; Heller, R.; Schmidtke, M.; Koch, M.; Löffler, B.; Ludwig, S.; Ehrhardt, C. Inhibition of Phosphatidylinositol 3-Kinase by Pictilisib Blocks Influenza Virus Propagation in Cells and in Lungs of Infected Mice. *Biomolecules* **2021,** *11, 808*. doi: 10.3390/biom11060808.
125. Hirata, N.; Suizu, F.; Matsuda-Lennikov, M.; Edamura, T.; Bala, J.; Noguchi, M. Inhibition of Akt kinase activity suppresses entry and replication of influenza virus. *Biochem. Biophys. Res. Commun.* ***2014,*** *450, 891-898.* doi: 10.1016/j.bbrc.2014.06.077.
126. Denisova, O.V.; Söderholm, S.; Virtanen, S.; Von Schantz, C.; Bychkov, D.; Vashchinkina, E.; Desloovere, J.; Tynell, J.; Ikonen, N.; Theisen, L.L.; Nyman, T.A.; Matikainen, S.; Kallioniemi, O.; Julkunen, I.; Muller, C.P.; Saelens, X.; Verkhusha, V.V.; Kainov, D.E. Akt inhibitor MK2206 prevents influenza pH1N1 virus infection in vitro. *Antimicrob. Agents Chemother.* **2014,** *58, 3689-3696.* doi: 10.1128/AAC.02798-13.
127. Bergmann, S.; Elbahesh, H. Targeting the proviral host kinase.; FAK.; limits influenza a virus pathogenesis and NFkB-regulated pro-inflammatory responses. *Virology* **2019,** *534, 54-63.* doi: 10.1016/j.virol.2019.05.020.
128. Bhatia, S.; Hilsch, M.; Cuellar-Camacho, J.L.; Ludwig, K.; Nie, C.; Parshad, B.; Wallert, M.; Block, S.; Lauster, D.; Böttcher, C.; Herrmann, A.; Haag, R. Adaptive Flexible Sialylated Nanogels as Highly Potent Influenza A Virus Inhibitors. *Angew. Chem. Int. Ed. Engl.* **2020**, *59,* 12417–12422. doi: 10.1002/anie.202006145.
129. Nie, C.; Stadtmüller, M.; Parshad, B.; Wallert, M.; Ahmadi. V.; Kerkhoff, Y.; Bhatia, S.; Block, S.; Cheng, C.; Wolff, T.; Haag, R. Heteromultivalent topology-matched nanostructures as potent and broad-spectrum influenza A virus inhibitors. *Sci. Adv.* **2021,** *7,* eabd3803. doi: 10.1126/sciadv.abd3803.