Binding of Influenza Viruses to Glycans

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Tadanobu Takahashi and Takashi Suzuki

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Abstract

All viruses require host cells in viral replication and have specificities of host (cell) ranges. Glycans on the cellular surface are highly diverse and species specific. Viral host specificities are often dependent on glycans expressed on the surface membranes of host cells. A representative life cycle of viral replication consists of receptor binding, entry, uncoating of viral capsid, synthesis of viral components (genomes and proteins), glycosylation of viral proteins, intracellular traffic of viral components, packaging of viral particles, and budding and release of progeny viruses on the cellular surface. In fact, since various viruses utilize specific glycans on the surface membranes of host cells as specific receptors, many researchers have studied virus binding of glycans to investigate the role of glycan as a viral receptor. Influenza viruses strongly bind to sialic acids existing at the terminal position of glycans and then initiate cell entry. Influenza viruses also have enzymes that destroy sialic acids, facilitating the release of progeny viruses from the surface membranes of infected cells and preventing

T. Takahashi • T. Suzuki (✉)
Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka-shi, Shizuoka, Japan
e-mail: takahasi@u-shizuoka-ken.ac.jp; suzukit@u-shizuoka-ken.ac.jp

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self-aggregation of progeny viruses through glycans on the viral surface glycoproteins. This report presents a review in terms of viral receptors about glycans recognized by influenza viruses.

Keywords
Hemagglutinin • Infection • Influenza virus • Receptor • Sialic acid • Sulfatide

Introduction

Representative orthomyxoviruses are influenza A, B, and C viruses (IAVs, IBVs, and ICVs), which are classified within the family *Orthomyxoviridae*. Influenza viruses are enveloped viruses with a diameter of 100 nm and respiratory pathogens presenting strong infection spread. IAVs and IBVs are eight-segmented single-stranded negative-sense RNA viruses. ICVs are seven-segmented single-stranded negative-sense RNA viruses. IAVs and IBVs cause severe cold symptoms by infection of the respiratory tract. ICVs generally cause mild cold symptoms. Viral hosts are wide species including humans, pigs, birds, and horses for IAVs and mainly humans for IBVs and ICVs. Host receptors of influenza viruses on the cellular surface membrane are sialic acid residues existing at the terminal position of glycans, *N*-acetylneuraminic acid (Neu5Ac) for IAVs and IBVs and *N*-acetyl-9-*O*-acetylneuraminic acid (Neu5,9Ac$_2$) for ICVs (Rogers et al. 1986; Suzuki et al. 1992). IAVs and IBVs have sialidase activity (an enzyme cleaving Neu5Ac from glyco-conjugates), and ICVs also have esterase activity (an enzyme cleaving 9-*O*-acetyl group from Neu5,9Ac$_2$) to prevent trapping of progeny viruses to sialic acid residues on the cellular surface and on the viral glycoproteins. These receptors containing sialic acids are thought to be gangliosides and/or glycoproteins.

Recognition by Influenza Viruses of Sialic Acid Linked to Galactose

Almost all human IAVs show preferential binding to Neu5Ac α2,6-linked to galactose (Neu5Acα2,6Gal), whereas almost all avian IAVs show preferential binding to Neu5Ac-α2,3 linked to galactose (Neu5Acα2,3Gal). Almost all swine IAVs bind to both Neu5Acα2,3Gal and Neu5Acα2,6Gal, equally or with predominance toward Neu5Acα2,6Gal (Ito et al. 1997; Suzuki et al. 1997). Almost all IBVs show preferential binding to Neu5Acα2,6Gal (Suzuki et al. 1992). IAVs and IBVs strongly recognize Neu5Acα2,6(or α2,3)Galβ1,3GlcNAc and Neu5Acα2,6(or α2,3)Galβ1,4GlcNAc through interactions of the viral surface glycoprotein, hemagglutinin (HA) (Suzuki et al. 1992; Suzuki 1994). Glycans possessing Neu5Acα2,6Gal are predominantly expressed in the human trachea. Glycans possessing both Neu5Acα2,3Gal and Neu5Acα2,6Gal are expressed in the pig trachea (Suzuki et al. 1997). Glycans possessing Neu5Acα2,3Gal are expressed in chicken eggs

Table 1 Glycans possessing sialic acids detected in host tracheas of IAVs

Host	Glycan
Human	Neu5Acα2,6Gal
Chicken	Neu5Acα2,3Gal
Horse	Neu5Gcα-R (R = aglycons)
	Neu5Gcα2,3Gal
Pig	Neu5Acα2,6Gal
	Neu5Acα2,3Gal
	Neu5Gcα2,3Gal

Neu5Gcα-R means that Neu5Gc residues are detected in the horse trachea

and the trachea (Table 1). Glycans recognized by respective IAVs coincide with respective virus replication sites expressing their glycans, strongly suggesting that their glycans are receptors of IAVs. In addition, IAVs also recognize the length and type of sugar chains on glycans. Human IAVs show stronger binding to a long chain, for example, a chain with many repeats of the lactosamine unit in polylactosamine possessing terminal Neu5Acα2,6Gal. In contrast, avian IAVs show stronger binding to a short chain, for example, a chain with less repeats of the lactosamine unit in polylactosamine possessing terminal Neu5Acα2,3Gal. Some H5N1 highly pathogenic avian IAVs (HPAIs) and H7N9 avian IAVs, isolated from humans, show increased binding activity to Neu5Acα2,6Gal. Acquisition of Neu5Acα2,6Gal binding activity of H5N1 HPAIs is one of the factors that lead to airborne transmission among ferrets (human infection and transmission model). Increased binding activity of avian IAVs and animal IAVs other than human IAVs to Neu5Acα2,6Gal could cause a pandemic of a new subtype IAV among humans. As an alternative pandemic mechanism, a new subtype IAV could arise by genetic reassortment among segmented viral RNAs from simultaneous infections of human and avian IAVs in pigs, which express both Neu5Acα2,3Gal (receptor of avian IAVs) and Neu5Acα2,6Gal (receptor of human IAVs) in the trachea. In this way, Neu5Ac binding properties of IAVs may be involved in the pandemic occurrence of a new subtype IAV. Since 2008, it has been reported that some IAVs, 2009 pandemic human H1N1 IAVs and avian IAVs including H5, H6, H7, and H9 subtypes, show preferential binding to 6-sulfo sialyl Lewis X. Terminal tri- or tetra-oligosaccharides of this glycan appear to be important for this binding. The function of the terminal structure of 6-sulfo sialyl Lewis X in IAV infection remains unknown.

Recognition by Influenza Viruses of Sialic Acid Species

Major sialic acids are classified into two types: Neu5Ac and *N*-glycolylneuraminic acid (Neu5Gc). Almost all equine IAVs show strong preferential binding to Neu5Gc α2,3-linked to galactose (Neu5Gcα2,3Gal) (Ito et al. 1997). Almost all avian IAVs also bind to Neu5Gcα2,3Gal, although their Neu5Gc binding activities

are weaker than their Neu5Ac binding activities (Ito et al. 1997, 2000). Some human and swine IAVs bind to Neu5Gc (preferentially to Neu5Gcα2,6Gal) (Suzuki et al. 1997; Takahashi et al. 2009). Glycans possessing Neu5Gc and Neu5Gcα2,3Gal are expressed in the horse trachea, duck intestine, and pig trachea, which are natural replication sites of IAVs (Suzuki et al. 1997; Ito et al. 2000). The function of Neu5Gc is predicted to be an IAV receptor, like Neu5Ac. There is a possibility that human and avian IAVs facilitate transmission to pigs through interactions with Neu5Gc. As described above, pigs are potential intermediate hosts that produce a new subtype IAV between human IAV and avian IAV. Neu5Gc binding properties of these IAVs may also be involved in pandemic occurrence.

Binding of Influenza Viruses to Glycans Containing No Sialic Acid

IAV specifically binds to sulfatide, even though it does not contain any sialic acids (Suzuki et al. 1996). Sulfatide is not an IAV receptor for initial infection, different from sialic acids. Sulfatide is 3-*O*-sulfated galactosylceramide, which is synthesized from galactosylceramide by cerebroside sulfotransferase. Sulfatide converts to galactosylceramide through the removal of the 3-*O*-sulfate group of sulfatide by arylsulfatase A. Sulfatide expression is controlled by cerebroside sulfotransferase and arylsulfatase A, distinctly different from the complex metabolism (initiation from glucosylceramide) of gangliosides. Apoptosis enhances IAV replication through enhancement of nuclear export of viral ribonucleoprotein complexes (vRNP). Sulfatide interacts with the newly synthesized HA transferred to the surface membranes of infected cells. The interaction of HA with sulfatide facilitates formation and replication of progeny virus particles through enhancement of nuclear export of vRNP by inducing caspase-3-independent apoptosis (Takahashi et al. 2008). The binding mechanism of HA ectodomain with sulfatide is thought to be different from that with Neu5Ac (Takahashi et al. 2013) (Table 2).

Conclusions and Perspectives

Influenza virus infects target tissues and/or organs of the host to produce progeny viruses. The viral binding specificity to the sugar chain structure in glycans is a critical factor in the viral target tissues and host ranges. IAVs of influenza viruses have a variety of host specificities including humans, birds, horses, and pigs and specifically infect and replicate in the trachea of humans, chickens, horses, and pigs. Avian IAVs preferentially bind to Neu5Acα2,3Gal, which is abundantly expressed in the chicken trachea. Human IAVs preferentially bind to Neu5Acα2,6Gal, which is abundantly expressed in the human trachea. Equine IAVs strongly bind to Neu5Gc, the content of which is nine times more than that of Neu5Ac in the equine trachea. Swine IAVs bind to Neu5Acα2,3Gal, Neu5Acα2,6Gal, and Neu5Gc residues, which are present in the pig trachea (Table 1). Binding specificities of IAVs to glycans possessing sialic acids coincide with infection and replication sites of IAVs

Table 2 Binding activities of influenza viruses to glycans

Virus	Glycan (references)
Human IAV	Neu5Ac (Neu5Gc) α2,6Gal (Suzuki et al. 1992; Suzuki 1994; Takahashi et al. 2009)
Avian IAV	Neu5Ac (Neu5Gc) α2,3Gal (Ito et al. 1997, 2000; Suzuki et al. 1992; Suzuki 1994)
Swine IAV	Neu5Acα2,6Gal (Suzuki et al. 1997)
	Neu5Ac (Neu5Gc) α2,3Gal (Suzuki et al. 1997)
Equine IAV	Neu5Gcα2,3Gal (Ito et al. 1997)
IAV	Sulfatide (Suzuki et al. 1996; Takahashi et al. 2008, 2013)
	Terminal tri- or tetra-saccharides of 6-sulfo sialyl Lewis X
IBV	Neu5Acα2,6Gal (Suzuki et al. 1992)
ICV	Neu5,9Ac_2 (Rogers et al. 1986; Suzuki et al. 1992)

in each host. This strongly suggests that receptor binding specificities of IAVs determine host specificities of IAVs. Shift of receptor binding specificities of IAVs may mean acquirement of transmission ability to other hosts. Cross-species transmission could cause a pandemic of a new subtype IAV in humans. Monitoring receptor binding specificities of avian and animal IAVs (especially HPAIs showing a mortality rate of approximately 60 % in humans) could be one of the means for controlling a future pandemic. The development of a highly sensitive and simple assay (suitable for high throughput) for determining receptor binding specificities of IAVs is needed.

IAVs also show strong binding to sulfatide. Progeny virus production in infected cells is induced by interaction of newly synthesized HA with sulfatide on the surface membranes of infected cells. Inhibitor of HA binding with sulfatide would be potential seeds of a novel anti-IAV drug to suppress progeny virus production in infected cells. Currently, specific sialidase inhibitors against IAV and IBV are being clinically used as commercial medicines. Some IAVs acquire resistance against some of these sialidase inhibitors. Although a vaccine against IAV and IBV is beneficial for preventing virus infection, it would not be efficient if a new subtype IAV causes a pandemic in humans because of the variety of antigenicities of surface proteins on IAV. All IAVs tested show strong binding to sulfatide. Inhibitors of HA binding with sulfatide would be more beneficial anti-IAV drugs against both sialidase inhibitor-resistant IAV and new pandemic IAV. To develop such an inhibitor, identification of the sulfatide binding site in HA and analysis of its binding mechanism at the molecular level are required. The interaction of HA with sulfatide also induces apoptosis and then facilitates nuclear export of vRNP in infected cells. Sulfatide is known to bind to many biological factors such as midkine, integrin, lamin, and bacterial and viral proteins. Thus, sulfatide is involved in various biological properties such as immune system, nervous system, kidney functions, insulin control, hemostasis/thrombosis, cancer, and other microbes. Further studies on sulfatide functions in IAV replication would contribute to elucidation of these biological mechanisms and diseases.

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