Virology 522 (2018) 37–45

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Receptor-binding properties of influenza viruses isolated from gulls

Alexandra S. Gambaryan[a,*], Tatyana Y. Matrosovich[b], Elisaveta Y. Boravleva[a], Natalia F. Lomakina[a,c], Svetlana S. Yamnikova[c], Alexander B. Tuzikov[d], Galina V. Pazynina[d], Nicolai V. Bovin[d], Ron A.M. Fouchier[e], Hans-Dieter Klenk[b], Mikhail N. Matrosovich[b,*]

[a] Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences, Moscow, Russia
[b] Institute of Virology, Philipps University, Marburg, Germany
[c] Gamaleya Scientific Research Institute of Epidemiology and Microbiology, Ministry of Health of the Russian Federation, Moscow, Russia
[d] The M.M. Shemyakin–Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia
[e] Department of Viroscience, Erasmus Medical Centre, Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Influenza
Hemagglutinin
Receptor specificity
Receptor-binding site
Sialic
Fucose
Aquatic birds
Transmission
Adaptation

ABSTRACT

Ducks, gulls and shorebirds represent the major hosts of influenza A viruses (IAVs) in nature, but distinctions of IAVs in different birds are not well defined. Here we characterized the receptor specificity of gull IAVs with HA subtypes H4, H6, H14, H13 and H16 using synthetic sialylglycopolymers. In contrast to duck IAVs, gull IAVs efficiently bound to fucosylated receptors and often preferred sulfated and non-sulfated receptors with Galβ1–4GlcNAc cores over the counterparts with Galβ1–3GlcNAc cores. Unlike all other IAVs of aquatic birds, H16 IAVs showed efficient binding to Neu5Acα2–6Gal-containing receptors and bound poorly to Neu5Acα2–3Galβ1–3-terminated (duck-type) receptors. Analysis of HA crystal structures and amino acid sequences suggested that the amino acid at position 222 is an important determinant of the receptor specificity of IAVs and that transmission of duck viruses to gulls and shorebirds is commonly accompanied by substitutions at this position.

1. Introduction

Wild aquatic birds of the orders *Anseriformes* (ducks, geese and swans) and *Charadriiformes* (gulls, terns and shorebirds) represent the major natural reservoirs of IAVs with various combinations of 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes (Olsen et al., 2006; Stallknecht and Shane, 1988; Webster et al., 1992). IAVs of aquatic birds occasionally transmit directly or indirectly to other hosts, such as gallinaceous poultry, horses, pigs and humans forming new stable host-specific lineages. Interspecies transmission of IAVs may require their adaptation to a spectrum of sialic acid-containing receptors and inhibitors in the target tissues of a new host. As a result, IAVs circulating in different species often differ by amino acid substitutions in the HA receptor-binding site (RBS) and have different receptor-binding specificities. For example, avian IAVs typically show a strong preference for Neu5Acα2–3Gal-terminated (avian-type) oligosaccharide receptors, equine viruses preferentially recognize Neu5Gcα2–3Gal-terminated receptors, and swine and human viruses bind to Neu5Acα2–6Gal-containing (human-type) receptors (for reviews, see (de Graaf and Fouchier, 2014; Matrosovich et al., 2008)). Thus, knowledge of the receptor distribution in different host species and of host-specific differences in the receptor specificity of IAVs is necessary to understand the molecular mechanisms underlying host-switching events.

Mallards and other species of dabbling ducks (*Anatinae*) carry all subtypes of IAVs other than H13 and H16 and show the highest virus prevalence among aquatic birds studied. Because of that, dabbling ducks are believed to play a key role in perpetuating IAVs in nature (Fouchier and Munster, 2009; Olsen et al., 2006). Despite significant divergent evolution of IAVs, viruses which primarily circulate in ducks show a marked conservation of the RBS and the receptor-binding specificity. They do not bind to Neu5Acα2–6Gal-containing receptors, they bind stronger to Neu5Acα2–3Galβ1–3-terminated (duck-type) receptors than to Neu5Acα2–3 Galβ1–4-terminated ones, and they do not tolerate fucosylation of the penultimate GlcNAc-3 moiety (for a review see ref. (Matrosovich et al., 2008)). IAVs with HA subtypes H5, H7 and H9, which circulate in land-based gallinaceous poultry display a different receptor-binding specificity. They typically show the highest binding avidity for Neu5Acα2–3Galβ1–4GlcNAc-terminated receptors containing fucose and/or sulfate at GlcNAc-3, and some of these IAVs in

* Corresponding authors.
E-mail addresses: al.gambaryan@gmail.com (A.S. Gambaryan), m.matrosovich@gmail.com (M.N. Matrosovich).

https://doi.org/10.1016/j.virol.2018.07.004
Received 26 April 2018; Received in revised form 3 July 2018; Accepted 5 July 2018

addition bind to Neu5Acα2–6Gal (Gambaryan et al., 2012, 2008; Yang et al., 2013b). These differences in receptor specificity correlate with limited data suggesting a dominance of non-fucosylated Neu5Acα2–3-Galβ1–3-terminated oligossacharides in intestinal epithelial tissues of ducks and on the presence of fucosylated, sulfated and non-modified Neu5Acα2–3Galβ1–4GlcNAc-containing receptors as well as Neu5Acα2–6 Gal-containing glycans in intestinal and respiratory tissues of chickens (Gambarian et al., 2006; Gambaryan et al., 2002; Guo et al., 2017; Hiono et al., 2014)

Gulls and terns (*Laridae;* called "gulls" for brevity throughout this paper) represent a distinctive avian host of IAVs (for reviews, see ref. (Arnal et al., 2015; Fouchier and Munster, 2009)). Thus, as influenza outbreaks usually occur in young gulls in densely populated colonies (Verhagen et al., 2014), features of IAV transmission in gulls may differ from features of waterborne virus transmission typical for ducks. Some species of gulls colonize urban areas and come in close contact to domestic animals and humans. Owing to the long distance migration, gulls seem to play a major role in intercontinental spread, reassortment and evolution of IAVs (Van Borm et al., 2012; Wille et al., 2011). In some regions, such as oceanic islands, gulls may be the major hosts for IAVs of different subtypes, including viruses commonly isolated from ducks (Lebarbenchon et al., 2015).

IAVs phylogenetically related to duck IAVs can be isolated from gulls, suggesting virus recent transmission between ducks and gulls. However, viruses with HA subtypes H13 and H16 are most frequently detected in gulls, but very rarely found in ducks. Furthermore, H13 and H16 subtype IAVs do not readily infect ducks under experimental conditions (Brown et al., 2012; Fereidouni et al., 2014). These findings indicate that H13 and H16 IAVs evolved into distinctive gull-adapted lineages and that a certain degree of host-range restriction exists between *Laridae* and *Anatinae* species. The detailed mechanisms of this restriction remain obscure.

We previously found that although duck and gull IAVs share preferential binding to Neu5Acα2–3Gal-terminated receptor moieties, they differ in the recognition of more distant oligosaccharide parts of the receptors (Gambaryan et al., 2005; Yamnikova et al., 2003). In this study, we wished to characterize receptor specificity of gull influenza viruses in more detail. To this end, we studied receptor-binding properties of a large panel of gull IAVs of subtypes H13 and H16 as well as of several other subtypes. We also looked for potential molecular mechanisms behind distinctive specificity of gull IAVs by analyzing published HA sequences and crystal structures.

2. Materials and methods

2.1. Viruses

Viruses described in the Table 1 were from the repositories of D.I. Ivanovsky Institute of Virology, Moscow, Russia; Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products, Moscow, Russia, and Erasmus Medical Center, Rotterdam, the Netherlands. Viruses A/Hong Kong/1/1968 (H3N2) and A/Mallard/Alberta/119/98 (H1N1) were provided by Earl Brown (University of Ottawa, Canada) and Robert Webster (St. Jude Children's Research Hospital, Memphis, TN, USA), respectively. Viruses were grown in 10 d embryonated chicken eggs. The allantoic fluids were clarified by low-speed centrifugation and used in the binding assays without further purification. Human IAVs A/NIB/23/1989-M (H1N1) and A/NIB/26/1990-M (H3N2) were provided by James Robertson (National Institute for Biological Standards and Control, Potters Bar, UK). These viruses were grown in MDCK cells.

To determine HA sequences, viral RNA was extracted from virus-containing allantoic fluid, reverse-transcribed and PCR-amplified using HA-specific primers. The PCR products were purified using the PCR purification Kit (Qiagen) and sequenced using the BigDye Terminator Cycle-Sequencing Ready Reaction (Applied Biosystems, CA).

Table 1
Influenza A viruses used in this study.

Virus	Subtype	Accession no.[a]	Residue[b]	
			193	222
Duck viruses				
Duck/France/46/1982	H1N1	N/A		
Mallard/Alberta/353/1988	H2N3	CY003936	T	K
Duck/Buryatiya/652/1988	H3N8	N/A		
Duck/Moscow/3554/2008	H3N1	GU991376*	N	W
Duck/Buryatiya/331/1978	H4N6	N/A		
Garganey/Kyrgyzstan/1047/1987	H4N6	EU564111	N	W
Shoveler/Buryatiya/1898/2000	H4N6	EU580566	N	W
Tufted duck/Buryatiya/1905/2000	H4N6	EU580567	N	W
Duck/Moscow/3558/2008	H4N6	GU991378*	N	W
Duck/Moscow/3641/2008	H11N9	GU991377*	D	K
H16, group 1[c]				
Black-headed gull/Turkmenistan/13/1976	H16N3	EU293864	N	G
Slender-billed gull/Astrakhan/28/1976	H16N3	EU293865	N	G
Little tern/Sweden/8897/2005	H16N3	KR087616	N	G
H16, group 2[c]				
Little tern/Gurjev/779/1983	H16N3	EU148601*	K	G
Black-headed gull/Netherlands/1/2007	H16N3	KR087609	K	G
Black-headed gull/Sweden/2/1999	H16N3	AY684888	K	G
H14				
Mallard/Gurjev/263/1982	H14N5	FLAH1424	D	R
Gull/ Gurjev/266/1982	H14N5	N/A		
Herring gull/Astrakhan/267/1982	H14N5	FJ975075	D	R
H6				
Duck/Moscow/3720/2009	H6N2	CY120771*	T	A
Gull/Moscow/3100/2006	H6N2	EU152237*	T	A
H13, group 1[c]				
Gull/Maryland/704/1977	H13N6	CY014694	T	G
Gull/Astrakhan/226/1984	H13N6	EU835895*	T	G
Great black-headed gull/Astrakhan/10/1988	H13N6	EU564106*	T	G
Black-headed gull/Astrakhan/44/1988	H13N6	EU564115*	T	G
Gull/Astrakhan/998/1990	H13N6	EU835896*	S	G
Gull/Astrakhan/3483/2002	H13N6	EU835897*	T	G
Black-headed gull/Netherlands/4/2007	H13N6	KR087580	T	G
H13, group 2[c]				
Gull/Astrakhan/1314/1979	H13N2	EU835898*	K	G
Great black-headed gull/Astrakhan/1421/1979	H13N2	EU293859	K	G
Great black-headed gull/Astrakhan/75/1983	H13N2	EU564107*	K	G
Little gull/Astrakhan/3357/2002	H13N6	EU564108*	R	G
Black-headed Gull/Sweden/55215/2006	H13N8	KR087597	K	G
Gull/Astrakhan/1846/1998	H13N6	EU580576	K	G
Black-headed gull/Netherlands/1/2000	H13N8	AY684886	K	G
Black-headed gull/Astrakhan/227/1984	H13N6	M26089	K	G
Great black-headed gull/Astrakhan/591/1982	H13N2	EU293860	K	G
Herring gull/Astrakhan/479/1985	H13N6	EU293863	K	G
Gull/Astrakhan/176/1986	H13N2	EU835899*	K	G
Gull/Astrakhan/1818/1998	H13N6	EU835900*	K	G
H4 gull viruses				
Common tern/Buryatiya/1901/2000	H4N6	EU580568	N	W
Gull/Buryatiya/2407/2001	H4N6	KP993204	N	L
Gull/Buryatiya/2408/2001	H4N6	KP993205	N	L
Gull/Astrakhan/3528/2002	H4N8	N/A		

[a] Genbank accession numbers for the HA nucleotide sequence. N/A, not available. Asterisks depict sequences determined in this study.
[b] Amino acid residue in positions 193 and 222 of the HA (H3 numbering).
[c] Groups 1 and 2 correspond to evolutionary lineages of H13 and H16 viruses shown in the Fig. 1.

2.2. Receptor binding assay

Receptor specificity of the viruses was characterized by determining their binding to soluble synthetic poly N-(2-hydroxyethyl)acrylamide-based sialylglycopolymers (SGPs) (GlycoNZ, Auckland, New Zealand). The SGPs contained 20 mol% of specific sialyloligosaccharide attached to the 30-kDa polymer. The structures and designations of the oligosaccharide moieties are shown below.