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Review article

Sialic acid tissue distribution and influenza virus tropism

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Abstract Avian influenza A viruses exhibit a strong preference for using α2,3-linked sialic acid as a receptor. Until recently, the presumed lack of this receptor in human airways was believed to constitute an efficient barrier to avian influenza A virus infection of humans. Recent zoonotic outbreaks of avian influenza A virus have triggered researchers to analyse tissue distribution of sialic acid in further detail. Here, we review and extend the current knowledge about sialic acid distribution in human tissues, and discuss viruses with ocular tropism and their preference for α2,3-linked sialic acid.

Keywords eye, influenza, receptor, sialic acid, tropism.

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Introduction

Influenza viruses, members of the family Orthomyxoviridae, are enveloped viruses with segmented, single-stranded, negative-sense RNA packaged in virions as ribonucleoproteins. Influenza viruses are further classified into three genera, A, B and C, on the basis of the antigenic diversity of virion proteins such as the matrix protein (M1) and nucleocapsid protein (NP). Further classification is based on the haemagglutinin (HA) and neuraminidase (NA) glycoproteins protruding from the viral envelope of influenza A and B viruses. Human influenza viruses mainly cause acute respiratory disease with high incidence and mortality, usually as epidemics but sometimes as worldwide pandemics. To date, 16 different influenza A virus HA subtypes (H1–H16) and nine different NA subtypes (N1–N9) have been identified in wild waterfowl, which are the major reservoir of all influenza A viruses. All mammalian influenza viruses are believed to originate from avian influenza A viruses, and only three subtypes of influenza A virus, H1N1, H2N2 and H3N2, have adapted to humans since 1918.[1] The causative viruses of the 1957 Asian (H2N2) and 1968 Hong Kong (H3N2) pandemics were found to be reassortants of human and avian influenza A viruses,[2,3] whereas the 1918 Spanish (H1N1) influenza A virus was likely to be entirely of avian origin but adapted to humans.[4] When H1N1 reappeared in 1977, H3N2 did not disappear but continued to circulate; therefore, currently, together with their H1N2 reassortant, three subtypes of influenza A virus are associated with epidemics. The ongoing zoonotic outbreaks of H5N1 avian influenza A virus have raised concerns that the situation may develop into a pandemic comparable in magnitude with the Spanish influenza virus pandemic.

Recently it become evident that other pathogenic viruses, including adenovirus type 37 (Ad37, family Adenoviridae), enterovirus type 70 (EV70, family Picornaviridae) and Newcastle disease virus (NDV, family Paramyxoviridae), all with a pronounced tropism for the human eye, share receptor preference with avian influenza A viruses for glycans terminating with α2,3-linked sialic acid (SAα2,3),[5–9] which is distinct from the SAα2,6-terminated receptor preferred by human influenza A viruses. Here, we review differences in sialic acid preferences of viruses that cause disease in humans and in the distribution of different sialic acids in tissues. Given the ocular tropism of Ad37, EV70 and NDV, and their preference for SAα2,3, we also discuss the human eye as a potential portal of entry for viruses such as the avian influenza A viruses.

Sialic acids, what and where?

Vertebrate cell surface glycans consist mainly of five- and six-carbon monosaccharides. One remarkable exception is the family of sialic acids, whose members have nine-carbon backbones.[10] Other important features of sialic acids are: (i) the high level of diversity – currently the family comprises over 50 different members, with neuraminic acid considered as the prototype, and includes important variants such as *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc) (Figure 1), (ii) their

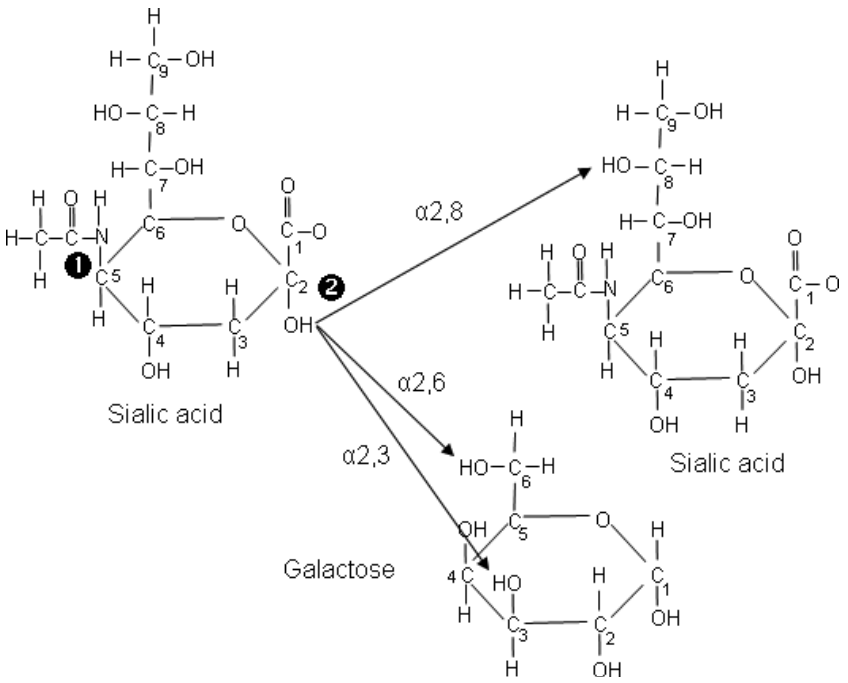

Figure 1. Sialic acid structure and glycosidic bonds to neighbouring saccharides. 1 corresponds to the site of N-linked acylation (glycolyl or acetyl), and 2 corresponds to the site of α2,3-, α2,6- and α2,8-glycosidic bonds between sialic acid and galactose or a neighbouring sialic acid sacharride.

presence mainly in higher animals and in certain bacteria, and (iii) their location, typically at the termini of glycan chains, and usually linked to galactose via α2,3- or α2,6-glycosidic bonds, or to sialic acid via α2,8-glycosidic bonds. Not surprisingly, their expression on the surface of cells makes sialic acids easily accessible as attachment molecules for a wide variety of microbial pathogens, including many viruses.

Until recently, little was known about the distribution of sialic acid in humans, although the expression of SAα2,6 on the surface of tracheal epithelial cells was demonstrated more than 15 years ago, which, at that time[11,12] correlated perfectly with the known receptor preference, tropism and host range of influenza A viruses. Thus, human influenza A viruses bind preferentially to cell surface SAα2,6 and replicate in the respiratory tract, whereas avian influenza A viruses bind preferentially to SAα2,3 and only recently were reported to target the human airway. In this context, it should be noted that humans, but not primates, express SAα2,6 on cells in the upper respiratory tract,[13] which is in agreement with the restricted replication of human influenza A virus in airways of chimpanzees.[14] Another important observation is that Neu5Gc is a frequent constituent of glycans in most birds and mammals, but the gene encoding the hydroxylase enzyme necessary for the conversion of Neu5Ac to Neu5Gc was inactivated in our human ancestors, but not in other primates, approximately 2·7 Ma,[15] possibly as a response to a microbial threat.[16] Some avian influenza A viruses replicate only or mainly in cells that express Neu5Gc.[17] Along with the current attention paid to sialic acid-binding viruses causing disease in humans, interest in sialic acid biology and its tissue distribution has intensified.

Recent research has more carefully documented the distribution of SAα2,3 and SAα2,6 in human tissues and more particularly in human airways. SAα2,3 is found on alveolar cells, whereas SAα2,6, with a few exceptions, is predominantly found on non-alveolar cells (Table 1). This correlates reasonably well with the predominant manifestations caused by human influenza A virus infection (rhinitis, pharyngitis, tracheobronchitis, bronchitis and bronchiolitis), indicating replication in the respiratory tract.[18] Importantly, the distribution of SAα2,3 and SAα2,6 also correlates with reports of lower respiratory tract symptoms and the difficulty in detecting H5N1 in nasopharyngeal samples,[19] and with the replication of H5N1 in cells lining the alveoli.[20] Specifically, SAα2,6 predominates on M cells in nasopharyngeal lymphoid tissue[21] and on epithelial cells in the nasal mucosa,[22] paranasal sinuses,[22] pharynx,[22] larynx,[23] trachea,[11–13,22–24] trachea/bronchi,[25,26] bronchi[22,24,27] and in bronchioles,[13,22] but not on alveolar cells.[24,28] Goblet cells in the larynx,[23] trachea[13,23] and bronchi[13,24] may also contain SAα2,6. Conversely, SAα2,3 is primarily found on alveolar cells[22,24,28] and on bronchiolar epithelial cells at the junction between bronchioles and alveoli,[22] and has been found on bronchial[27] and tracheal[24] epithelial cells. Epithelial tracheobronchial cells[25,26] and epithelial cells in the nasal mucosa[22] may also express SAα2,3, albeit at lower levels. Goblet cells in the larynx,[23] trachea[11–13,23] and bronchial[13] epithelium also contain SAα2,3. Nasopharyngeal and bronchial epithelial

Table 1. Distribution of SAα2,3 or SAα2,6 on cells in human airways and eyes*

Tissue	Glycan	Reference
Human airways		
M cells in nasopharyngeal lymphoid tissue	SAα2,6	21
Nasal mucosa	SAα2,6	22
Paranasal sinuses	SAα2,6	22
Pharynx	SAα2,6	22
Larynx	SAα2,6	23
Trachea	SAα2,6	11–13,22–24
Trachea/bronchi	SAα2,6	25,26
Bronchi	SAα2,6	22,24,27
Bronchioles	SAα2,6	13,22
Laryngeal, tracheal and bronchial** goblet cells	SAα2,6	13,23,24
Alveoli	SAα2,3	22,24,28
Alveoli	SAα2,3Gal β1,4GlcNAc	28
	SAα2,3Gal β1,3GalNAc	28
Bronchiolar cells junctioning bronchiole and alveoli	SAα2,3	22
Bronchi	SAα2,3	27
Bronchi	SAα2,3Gal β1,4GlcNAc	28
Trachea/bronchi**	SAα2,3	25,26
Trachea	SAα2,3	24
Nasopharynx	SAα2,3Gal β1,4GlcNAc	28
Nasal mucosa**	SAα2,3	22
Laryngeal, tracheal and bronchial** goblet cells	SAα2,3	11–13,23
Human eyes		
Conjunctiva	SAα2,3Gal β1,4(Fucα1,3)GlcNAc	38
	SAα2,3Gal β1,3(Fucα1,4)GlcNAc	39
Transformed conjunctival cells	SAα2,3(6)	40,71

*Cells are of epithelial origin if not otherwise stated.
**Expression is stated to be relatively low or on few cells in corresponding reference(s).

cells abundantly express SAα2,3 linked via galactose to *N*-acetylglucosamine (SAα2,3Galβ1,4GlcNAc), but not SAα2,3 linked via galactose to *N*-acetylgalactosamine (SAα2,3Galβ1,3GalNAc).[28] Both trisaccharides, however, are abundantly expressed on alveolar pneumocytes.

The role of ciliated versus non-ciliated cells as targets for influenza A viruses has also been investigated. In the upper respiratory tract, SAα2,6 seems to be expressed on both ciliated and non-ciliated cells, whereas SAα2,3 tends to be expressed mainly on ciliated cells. Specifically, SAα2,6 is found not only on non-ciliated tracheal cells,[24] and non-ciliated tracheobronchial cells,[25,26] but also on ciliated cells in the upper respiratory tract,[28] ciliated tracheal cells,[11,12,24] ciliated bronchial cells,[24] the ciliated borders of bronchial epithelial cells[13] and to a lesser extent on ciliated tracheobronchial cells.[25,26] SAα2,3 has been reported to be expressed on ciliated nasopharyngeal,[28] ciliated tracheal,[24] ciliated tracheobronchial cells[25,26] and ciliated bronchial cells,[24,28] and also on non-ciliated cuboidal bronchial cells at the junctions between bronchioles and alveoli.[22] Thus, the distribution of SAα2,3 and SAα2,6 in human cells and tissues (Table 1) generally parallels the cell and tissue tropism of influenza A viruses, [22,25,26,28,29] and emphasizes sialic acid's role as a critical determinant of influenza A virus tropism.

SAα2,3-binding viruses and ocular tropism

With few exeptions, sialic acid-binding viruses isolated from humans exhibit a clear preference for two organs, the respiratory tract and the eye (Table 2). SAα2,6-binding viruses, such as human influenza A and B viruses, coronavirus OC43, polyomavirus JC and human parainfluenza virus 3, are more frequently isolated from the respiratory tract than from the eye. The human eye, on the other hand, apparently constitutes a very efficient replication site for at least three unrelated viruses with specificity for SAα2,3. Ad37 was isolated for the first time in 1976[30] and has been demonstrated to be one of the most frequent agents of epidemic keratoconjunctivitis (EKC). [31] The large amounts of Ad37 detected in eye swabs from EKC patients[32] are consistent with the observations that Ad37 is readily transmitted via direct or indirect contact[33] and that EKC is common in densely populated areas of the world.[34] EV70 was first isolated during a pandemic of a highly contagious form of acute haemorrhagic conjunctivitis (AHC) that originated in Ghana in 1969[35] and subsequently spread through Africa to Asia. EV70 was also responsible for a second pandemic of AHC in the early 1980s[36] that included both the eastern and western hemispheres, and for numerous regional outbreaks. Like Ad37, EV70 also uses SAα2,3 as a cellular receptor.[7] The third virus that binds to SAα2,3 is NDV,[9] a virus of domestic fowl that has been associated with several zoonotic outbreaks of conjunctivitis among poultry and kitchen workers and with case reports of laboratory infection.[37] The ability of these three viruses (Ad37, EV70 and NDV) to utilize the eye as a primary site of replication further emphasizes the potential role of the eye as a gateway for infections caused by other viruses that use SAα2,3 as a receptor. Therefore, it is important to highlight the potential of ocular SA, in general, as a receptor and of ocular SAα2,3 in particular, as a primary target of previously unrecognized importance for human infection by other viruses, including avian influenza A virus.

Table 2. Viruses with specificity for SAα2,3 or SAα2,6 and their tropism in humans

Virus	Specificity	Tropism in humans	References
Adenoviridae			
Ad37	SAα2,3	Ocular	6,31
Picornaviridae			
EV70	SAα2,3	Ocular (respiratory, CNS)	7,36,45
CVA24v	SAα2,3/6	Ocular, respiratory	40
Reoviridae			
Reo1	SAα2,3	Intestinal (ocular, CNS)	72
Paramyxoviridae			
PIV1	SA2,3	Respiratory	27
PIV3	SAα2,3/6	Respiratory(CNS)	73,74
PIV5	SAα2,3	Respiratory	75
NDV	SAα2,3	Ocular (CNS)	9
Orthomyxoviridae			
Avian influenza A H7	SAα2,3	Ocular (respiratory)	56,76
Avian influenza A H5	SAα2,3	Respiratory, ocular (CNS)	42,44,58,68
Human influenza A, B	SAα2,6	Respiratory (ocular, CNS)	8,18,77
Coronaviridae			
OC43	SAα2,6	Respiratory (CNS)	78,79
Polyomaviridae			
JC	SAα2,6	Respiratory (lymphoid, renal, CNS)	80,81
BK	SAα2,3	Respiratory, renal (ocular, CNS)	80,82,83

Ad37: adenovirus 37; EV70: enterovirus type 70; CVA24v: coxsackievirus A 24 variant; Reo1: reovirus 1; PIV1,3,5: parainfluenzavirus 1, 3, 5; NDV: Newcastle disease virus; OC43, JC, BK: strain OC43, strain JC and strain BK respectively.

It should also be noted that the human eye is an organ in which many other viruses replicate. For example, herpes simplex virus, measles virus, respiratory syncytial virus, echoviruses and species B adenoviruses have all been isolated from the human eye, but use cellular receptors other than SA; therefore, they are not discussed further in this review. For other SAα2,3-binding viruses with respiratory tropism, such as human parainfluenza viruses and polyomavirus BK, it will also be of interest to identify the cells in which these viruses replicate, and to determine if these cells express SAα2,3.

Sialic acid in the human eye

Relatively little is known about the levels and distribution of sialic acid on cells in the human eye. Sialyl Lewis X (sLe^x; SAα2,3Galβ1,4(Fucα1,3)GlcNAc) and Sialyl Lewis A (sLe^a; SAα2,3Galβ1,3(Fucα1,4)GlcNAc) have both been identified on conjunctival epithelial cells using antibodies,[38,39] and both SAα2,3 and SAα2,6 have been detected on transformed, normal human conjunctival epithelial cells (NHC) using lectins.[40] Thus, the presence of SAα2,3 on conjunctival cells is in agreement with the ocular tropism of SAα2,3-binding viruses such as Ad37, EV70 and NDV. This also suggest that human conjunctival cells are susceptible to both human and avian influenza A viruses, which may be of importance when considering potential sites where recombinant influenza A viruses with pandemic potential may be generated. The role of a 'mixing vessel' has previously been allotted to the pig,[41] an animal known to express both SAα2,3 and SAα2,6 in the respiratory tract and susceptible to both human and avian influenza A viruses. The presence of both SAα2,3 and SAα2,6 in human conjunctival cells indicates that these cells should not be ignored as candidate mixing vessels.

Avian influenza A virus infections in humans

A major zoonotic outbreak caused by avian influenza A virus occurred in 1997, when at least 18 people in Hong Kong were infected by the H5N1 virus and six of these 18 individuals died, mainly due to respiratory failure.[42] Since 2003, recurrent outbreaks of zoonotic H5N1 virus have been reported from Asia and Africa, where this subtype may now be enzootic.[43] Recently, it has become evident that not only human influenza A virus but also avian influenza A virus infection may give rise to serious CNS manifestations.[44] In this context, it is interesting that several viruses that replicate in the human eye (including EV70 and NDV) have also been associated with CNS complications,[45] suggesting that the CNS is an accessible secondary target for SAα2,3-binding viruses that replicate in the human eye.

Is the eye a potential portal of entry for avian influenza A viruses?

Multiple reports describe the eye as an intial site of replication in humans for measles virus,[46] respiratory syncytial virus,[47] and CVA24v,[48] and in experimentally infected animals for ebola virus, [49] human influenza A virus,[50] and importantly, avian influenza A virus including H5N1,[51] followed by further transmission to non-ocular sites, including airways. Conjunctivitis caused by H7 subtypes of avian influenza A virus has been reported sporadically over the years,[52–55] and both before and after the zoonotic outbreak of H7N7 in the Netherlands in 2003.[56] During this outbreak, one person died of respiratory failure; however,

conjunctivitis was the major manifestation observed. It was recently demonstrated that virus isolated from this outbreak was capable of direct binding to human conjunctival tissue.[57] H7N7 influenza virus replication in the eyes of patients was high, and similar to the level of replication of cocirculating human influenza H3N2 virus in patients with respiratory tract infection.[56] While it seems clear that the human eye is the initial and main site of replication for some H7 viruses, this is not obvious for other avian influenza A viruses. While avian H5N1 viruses replicate in the respiratory tract, mainly in alveolar cells[20] that express SAα2,3,[22] the potential for H5N1 influenza A virus replication in the eye needs further attention. H5N1 was reported to be associated with conjunctivitis during the outbreaks in Hong Kong in 1997[42] and in Turkey in 2005–2006.[58] Importantly, 14/37 (37%) of Egyptian patients with H5N1 exhibited conjunctivitis.[59] Additional information indicates that the eye may be an initial site of replication for H5N1 virus in humans. For example, a Belgian veterinarian who investigated two H5N1-positive Thai eagles developed conjunctivitis 2 days after examination of the eagles, but no respiratory symptoms.[60] This raises the question whether H5N1 may use the human eye as a portal of entry preceeding transmission to the respiratory tract.[5] This possibility is supported by the relatively longer incubation period in humans for H5N1 avian influenza A virus (normally 4–7 days)[58] than for human H3N2 (normally 1–4 days),[18] avian H7N3 (1–3 days)[61] and avian H7N7 (1 day),[62,63] as well as the low frequency of virus detection and the relatively low viral load in nasopharyngeal samples from zoonotic H5N1 patients.[19] Also consistent with this idea is that one of the Turkish patients who developed respiratory symptoms of zoonotic H5N1 in the 2005–2006 outbreak presented with conjunctivitis.[58,64] Recent work in animal models has confirmed that avian H7 viruses that replicate in mouse eyes (but not H5 or human H3 viruses) cause lethal disease following ocular infection of mice.[65] At present, it is unclear why H5 viruses are much less frequently associated with conjunctivitis in humans than are H7 viruses. Glycan arrays can be used to identify sialylated glycans recognized by influenza virus and other viruses.[66–68] A recent study using glycan arrays[66] showed differences in the sialic acid-binding specificity of influenza virus H7 isolates. European and North American isolates associated with conjunctivitis exhibited a strong preference for binding SAα2,3; however, the pattern of binding for the European isolates more closely paralleled binding of classic avain influenza viruses, while the North American isolates showed reduced binding to glycans containing SAα2,3 and increased binding to glycans containing SAα2,6. Virus binding was skewed even more towards glycans containing SAα2,6 for North American H7 isolates that were not associated with conjunctivitis. Glycan array analysis will be an important tool for unravelling the complex inter-relationship between sialic acid-binding specificity and influenza virus tropism and pathogenicity. However, for identification of the specific sialic acid-containing cellular receptors used by influenza A viruses, additional experimental approaches are required.

The possibility exists that influenza virus replication in the human eye may occur asymptomatically. It has been suggested that asymptomatic replication occurred frequently during the zoonotic outbreak of H7N7 in the Netherlands in 2003[69] and during the zoonotic outbreaks of H5N2 in Japan in 2005 (http://www.mhlw.go.jp/english/topics/influenza/avian02.html) and H5N1 in South Korea in 2006 (http://www.promedmail.org/pls/otn/f?p=2400:1202:857450566246460::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,34431). Whether or not patients or their relatives were asked about a history of conjunctivitis during outbreaks of H5N1 has not been reported, and there is no evidence that ocular samples from infected patients or from individuals exposed to H5N1 were tested for the presence of virus. Moreover, human-to-human transmission of avian H5N1 influenza A virus is inefficient,[70] which is compatible with virus replication that is limited to the lower respiratory tract,[20] and there are no reported cases of human-to-human transmission by small-particle aerosols.[70] However, influenza infection in the human eye represents a potential source for transmission between humans, but not necessarily a signal of an imminent pandemic. Mild or subclinical ocular infections may explain the prolonged incubation time before respiratory symptoms appear in zoonotic H5N1 cases, and raise the concern that unrecognized cases with mild or inapparent respiratory infection is common.

Conclusions

Not long ago, differences in the receptor specificity of human and avian influenza A viruses and a presumed lack of sialic acid receptors for avian influenza A viruses in humans was believed to constitute an almost absolute barrier to human infection by avian influenza viruses. Recent zonootic outbreaks of avian influenza A viruses triggered research on sialic acid distribution in both humans and birds, leading to evidence that this early picture was invalid. It is now clear that SAα2,3 is expressed on both alveolar and conjunctival cells in humans, which are important targets for viruses such as avian influenza A virus. From these findings it can be concluded that sialic acid is a critical determinant of influenza A virus tropism and that the human eye is a potential portal of entry for emerging sialic acid-binding viruses. However, further investigation of sialic acid distribution and more extensive diagnostic efforts, which should include ocular sampling, are required in

order to better understand the tropism and transmission of zoonotic influenza A viruses.

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