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Influenza A and Sendai viruses preferentially bind to fucosylated gangliosides with linear poly-*N*-acetyllactosaminyl chains from human granulocytes

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Abstract

Influenza A and Sendai viruses are known to bind to various extent to neolacto-series gangliosides IV^{3}Neu5Ac-nLcOse$_4$Cer, IV^{6}Neu5Ac-nLcOse$_4$Cer, and VI^{3}Neu5Ac-nLcOse$_6$Cer, which are the dominant gangliosides of human granulocytes. Recently, minor gangliosides of granulocytes were characterized and found to express sialyl Lewis^x and VIM-2 epitopes. These long chain linear monosialogangliosides with nLcOse$_8$, and nLcOse$_{10}$, cores, carrying one to three fucoses, are shown in this study to bind with strong avidity to influenza A/PR/8/34 (H1N1), A/X-31 (H3N2), and Sendai virus (Z-strain) using the overlay technique. These and recent data from other groups imply that selectins and virus hemagglutinins are capable of competing with lipid bound sialyl Lewis^x and VIM-2 epitopes on myeloid cells during inflammatory reactions. © 1996 Elsevier Science Ltd.

Keywords: Gangliosides; Glycosphingolipids; Receptor function; Sialyl Lewis^x; VIM-2; Virus binding

Abbreviations: GSLs, glycosphingolipids; Neu5Ac, *N*-acetylneuraminic acid [33]; Sialyl Lewis^x determinant, Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc-R; VIM-2 epitope, Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAc-R. The designation of the glycosphingolipids follows the IUPAC-IUB recommendations [34]. nLcOse$_4$Cer, Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer; nLcOse$_6$Cer, Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer; nLcOse$_8$Cer, Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-3)$_2$Galβ1-4Glcβ1-1Cer; nLcOse$_{10}$Cer, Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-3)$_3$Galβ1-4Glcβ1-1Cer; G_{M3} according to Svennerholm [35].

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1. Introduction

Gangliosides are distinguished from other glycosphingolipids (GSLs) by the presence of sialic acid, a characteristic carbohydrate constituent [1]. Structures and functions of gangliosides have been widely reviewed [2–4]. They act, e.g. as receptors for toxins and bacteria [5], viruses [6,7], and other ligands, and are known to be involved in cell–cell-recognition phenomena [8,9]. Terminally sialylated gangliosides are important receptor binding sites for viruses [10] and are able to modulate virus attachment [11]. To investigate the accessibility of viruses to cells, several assay systems have been developed to prove the adsorption to [12], fusion with [13], or infection of target cells [14]. Solid phase binding assays in microtitre wells [15] and the overlay technique [16,17] are well established tools. In a previous work we showed the virus receptor function of neolacto-series gangliosides IV^3Neu5Ac-nLcOse$_4$Cer, IV^6Neu5Ac-nLcOse$_4$Cer, and VI^3Neu5Ac-nLcOse$_6$Cer, isolated from human granulocytes, to influenza A and Sendai virus [18]. In this study, the preferential binding of both types of virus towards fucosylated gangliosides with linear poly-*N*-acetyllactosaminyl chains, carrying sialyl Lewis^x and VIM-2 motifs, is reported.

2. Experimental

Gangliosides from human granulocytes.—The isolation of gangliosides from human granulocytes was performed according to standard procedures as recently reported [18]. Briefly, GSLs were extracted with chloroform–MeOH mixtures, partitioned according to Folch et al. [19], and gangliosides of upper phases were obtained by anion exchange chromatography on DEAE sepharose. Phospholipids were saponified with 1 N NaOH, and the gangliosides were further purified by Iatrobeads chromatography according to Ueno et al. [20]. The isolation and purification of gangliosides of fractions designated with X, Y, and Z was performed by TMAE-Fractogel (Merck, Darmstadt), silica gel, and reversed phase HPLC as recently published [21].

Monoclonal antibodies.—The sialyl Lewis^x antigen recognizing monoclonal antibody CSLEX1 [22] was obtained from Becton Dickinson (Heidelberg). The VIM-2 specific antibody CDw65 [23] was from Dianova (Hamburg). TLC-immunostaining of gangliosides of fractions X, Y, and Z with CSLEX1 and CDw65 monoclonal antibodies was carried out as recently described [21].

Thin-layer chromatography.—Gangliosides were separated on glass-backed silica gel 60 precoated HPTLC plates (size 10 cm × 10 cm, thickness 0.2 mm, E. Merck, Darmstadt) in chloroform–MeOH–H_2O (120–85–20, by vol) containing 2 mM $CaCl_2$ and visualized by resorcinol according to Svennerholm [24].

Viruses.—Human influenza virus A/PR/8/34 (H1N1) and a reassortant between A/PR/8/34 and A/Aichi/2/68, A/X-31 (H3N2) were propagated in embryonic chicken fibroblasts and Sendai virus (HNF1, Z-strain) was grown in MDCK cells [25]. Metabolic labelling with L-[^{35}S]-methionine was carried out from 5 to 24 h after infection. Progeny virus was purified by adsorption to and elution from chicken

erythrocytes and subsequent centrifugation for 90 min at 120 000 *g*. The sediment was resolved in 1 mL phosphate buffered saline (PBS).

TLC overlay technique.—Binding assays of viruses to methacrylate fixed gangliosides on silica gel precoated plates were performed according to Magnani et al. [26] with some modifications. After chromatography the plates were thoroughly dried for 0.5 h over P_2O_5 under vacuum in a desiccator and the silica gel was then fixed by chromatography in hexane saturated with polyisobutylmethacrylate (Plexigum P28, Röhm, Darmstadt). The plates were cut with a diamond cutter into 1.5 cm × 10 cm strips per lane, which were then soaked for 15 min in 1% bovine serum albumin in PBS to block unspecific binding. The solution was thoroughly withdrawn by suction and 80 μL of radiolabelled virus suspension was applied per lane (about 2×10^5 cpm). The strips were covered with small pieces of parafilm™ and incubated in a humidified atmosphere for 2 h at +4 °C. After incubation, the virus suspension was tipped off and the plate was washed six times with PBS. The dried plate was exposed to Hyperfilm™-^{3}H (Amersham Buchler, Braunschweig) for 20 days at +4 °C.

TLC scanning.—Stained ganglioside chromatograms and virus autoradiographies were scanned with the Desaga CD 60 scanner (Heidelberg) equipped with an IBM AT compatible personal computer and densitometric software. Intensities of resorcinol stained bands and autoradiographies were measured in reflectance mode at 580 nm with a light beam slit of 0.1 mm × 2 mm. Ratios of bound ^{35}S-labelled virus to gangliosides were calculated from the average of three scans from each spot.

3. Results

Gangliosides from human granulocytes.—The major gangliosides of human granulocytes are G_{M3}(Neu5Ac) and the neolacto-series gangliosides IV^{3}Neu5Ac-nLcOse$_4$Cer, IV^{6}Neu5Ac-nLcOse$_4$Cer, and VI^{3}Neu5Ac-nLcOse$_6$Cer [18,27]. The resorcinol stained thin-layer chromatogram is shown in Fig. 1 (lane a). Ganglioside pairs differ in their respective ceramide portions, composed mainly of C_{24}- and C_{16}-fatty acids and in some cases substituted to a minor extent by C_{22}-fatty acid. The minor ganglioside fractions of human granulocytes, designated with X, Y, and Z (Fig. 1, lane a), are composed of fucosylated monosialogangliosides with linear poly-*N*-acetyllactosaminyl chains, which have been recently structurally characterized [21]. Their structures and binding specificities towards sialyl Lewis^x (CSLEX1) [22] and VIM-2 epitopes (CDw65) [23] are listed in Table 1, the antibody binding epitopes in Table 2. Three gangliosides were found to carry a terminal sialyl Lewis^x group and five are characterized by the VIM-2 structure, which differs in the inner chain fucosylation compared to the sialyl Lewis^x antigen.

Virus binding to major gangliosides of human granulocytes.—The two influenza A virus strains (A/PR/8/34 and A/X-31) and Sendai virus (Z-strain) exhibit specific adhesion to terminal Neu5Ac α2-3Gal and/or Neu5Ac α2-6Gal sequences (Fig. 1, lanes b–d), also influenced by the chain length of the ganglioside carbohydrate backbones [18]. A/PR/8/34 bound all gangliosides from human granulocytes to various extent (Fig. 1, lane b). Relative binding capacities (see Table 3) indicate a preferential binding to α2-3 linked Neu5Ac as in Neu5Ac-LacCer, IV^{3}Neu5Ac-nLcOse$_4$Cer, and

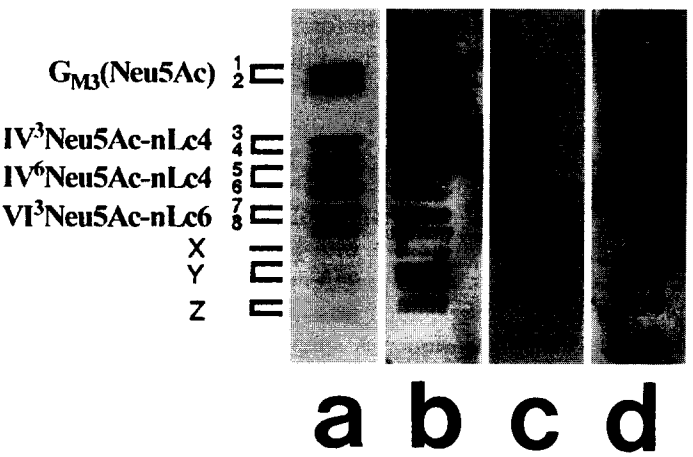

Fig. 1. Autoradiographies of TLC overlay assays of gangliosides from human granulocytes with ^{35}S labelled influenza A/PR/8/34 (lane b), A/X-31 (lane c) and Sendai virus (lane d), exposure time 20 d. The parallel resorcinol stain is shown in lane a. In each lane 20 μg of gangliosides were applied and chromatographed in chloroform–MeOH–H_2O (120–85–20, by vol) with 2 mM $CaCl_2$. The minor gangliosides of fractions X, Y, and Z are depicted in Table 1. nLc4 = $nLcOse_4Cer$; nLc6 = $nLcOse_6Cer$.

VI^{3}Neu5Ac-$nLcOse_6Cer$ compared with α2-6 linked Neu5Ac as in IV^{6}Neu5Ac-$nLcOse_4Cer$. This is in agreement with previous studies of Suzuki et al. [28]. Influenza virus strains exhibit markedly different specificities for receptor determinants [6,29], also found in this study for gangliosides from human granulocytes. In contrast to A/PR/8/34, strain A/X-31 preferentially bound to α2-6 linked Neu5Ac (Fig. 1, lane c), as shown for the IVNeu5Ac-$nLcOse_4Cer$ isomers (Table 3). However, A/X-31 also bound to α2-3 linked Neu5Ac if presented on a longer backbone as in VI^{3}Neu5Ac-$nLcOse_6Cer$. This high binding could be due to a more accessible presentation of the ligand determinant on the TLC plate. This is in contrast to A/PR/8/34 which

Table 1
Structures of minor gangliosides from human granulocytes

Ganglioside fraction	Structure	Fatty acid	CSLEX1 [a] (Sialyl Le^x)	CDw65 [a] (VIM-2)
X	VI^{3}Neu5Ac,V^{3}Fuc-$nLcOse_6Cer$	C_{16}	+	−
Y	VIII^{3}Neu5Ac,V^{3}Fuc-$nLcOse_8Cer$	C_{24}	−	+
	VIII^{3}Neu5Ac,V^{3}Fuc-$nLcOse_8Cer$	C_{16}	−	+
	VIII^{3}Neu5Ac,V^{3}Fuc,III^{3}Fuc-$nLcOse_8Cer$	C_{16}	−	+
	VIII^{3}Neu5Ac,VII^{3}Fuc,V^{3}Fuc-$nLcOse_8Cer$ [b]	C_{16}	+	−
Z	VIII^{3}Neu5Ac,VII^{3}Fuc,V^{3}Fuc,III^{3}Fuc-$nLcOse_8Cer$	C_{16}	+	−
	X^{3}Neu5Ac,VII^{3}Fuc-$nLcOse_{10}Cer$	C_{16}	−	+
	X^{3}Neu5Ac,VII^{3}Fuc,V^{3}Fuc-$nLcOse_{10}Cer$ [b]	C_{16}	−	+

[a] Monoclonal antibody binding; data drawn from [21].
[b] Proposed structure; internal fucosylation at position III instead of position V can not be excluded.

Table 2
Binding specificities of monoclonal antibodies CSLEX1 and CDw65

Monoclonal antibody	Epitope
CSLEX1 (Sialyl Le^x)	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc- 3 ↑ 1 Fucα
CDw65 (VIM-2)	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Gal- 3 ↑ 1 Fucα

preferentially bound to gangliosides with α2-3Neu5Ac linked to the nLcOse$_4$ backbone. Sendai virus (Fig. 1, lane d) also clearly preferred gangliosides with longer backbones (Table 3). But in contrast to influenza A/X-31, Sendai virus did not bind to α2-6 linked Neu5Ac-containing structures. Compared to the major gangliosides 1 to 8, all three viruses showed preferential binding to the minor ganglioside fractions X, Y, and Z (commented on below).

Virus binding towards ganglioside fraction X.—The two influenza A virus strains and Sendai virus all bind to the VI^{3}Neu5Ac,V^{3}Fuc-nLcOse$_6$Cer ganglioside. Despite the low expression of this ganglioside in human granulocytes (compared to G$_{M3}$(Neu5Ac) and the major gangliosides with nLcOse$_4$- and nLcOse$_6$-cores), this ganglioside with

Table 3
Relative binding capacities of influenza A and Sendai viruses to major gangliosides from human granulocytes revealed by the TLC overlay technique

Ganglioside No. [a]	Major fatty acid	Structure	Relative binding [b]		
			Influenza A/PR/8/34	Influenza A/X-31	Sendai virus
1	$C_{24:1}$	II^{3}Neu5Ac-LacCer	5.4	0.0	0.0
2	$C_{16:0}$	II^{3}Neu5Ac-LacCer	5.6	0.0	0.0
3	$C_{24:1}$	IV^{3}Neu5Ac-nLcOse$_4$Cer	9.9	0.0	0.8
4	$C_{16:0}$	IV^{3}Neu5Ac-nLcOse$_4$Cer	8.2	0.0	1.1
5	$C_{24:1}$	IV^{6}Neu5Ac-nLcOse$_4$Cer	1.0	1.0	0.0
6	$C_{16:0}$	IV^{6}Neu5Ac-nLcOse$_4$Cer	1.8	1.8	0.0
7	$C_{24:1}$	VI^{3}Neu5Ac-nLcOse$_6$Cer	4.6	4.9	3.2
8	$C_{16:0}$	VI^{3}Neu5Ac-nLcOse$_6$Cer	4.5	7.1	4.6

[a] According to Fig. 1.
[b] Ratio of bound ^{35}S-labelled viruses to gangliosides, calculated from the intensities of autoradiographies and resorcinol stained bands by densitometric scanning.

sialyl Lewis^x structure shows strong virus binding capacity (Fig. 1). Thus, fucosylation of this monosialoganglioside at penultimate position V of the nLcOse$_6$Cer did not abolish binding, but seems to enhance virus adhesion.

Virus binding towards ganglioside fraction Y.—This fraction was found to be homogeneous by the fact that all gangliosides bear the nLcOse$_8$ core but differ in number and attachment sites of fucoses. Further heterogeneity is due to variability in the ceramide portions of the monosialogangliosides carrying C_{24}- and C_{16}-fatty acids. Fraction Y contains as major compounds three gangliosides with VIM-2 epitopes and one sialyl Lewis^x bearing GSL in low quantity (Table 1). The avidity of virus binding to this fraction declined in the order A/PR/8/34 > Sendai > A/X-31 virus. However, all three strains displayed clear recognition of sialyl Lewis^x and/or VIM-2 epitopes, also suggesting a positive influence of fucosylation upon virus attachment.

Virus binding towards ganglioside fraction Z.—The heterogeneity in this fraction is caused by the presence of long-chain gangliosides with nLcOse$_8$Cer and nLcOse$_{10}$Cer structures, also differing in their respective fucosylation patterns. The Z fraction is composed of one sialyl Lewis^x and two VIM-2 bearing gangliosides (Table 1). A/PR/8/34 virus showed the most intensive binding towards these ligands, followed by Sendai virus, whereas only traces of bound A/X-31 virus could be detected on the autoradiography (Fig. 1). However, fucosylated monosialogangliosides of the Z fraction represent strong binding ligands for influenza A and Sendai viruses.

4. Discussion

In this study the high avidity of two influenza A and one Sendai virus strain towards fucosylated long chain linear polylactosaminyl gangliosides with sialyl Lewis^x and VIM-2 epitopes could be clearly demonstrated. Also, these compounds are only expressed in low amounts and are hardly detectable on HPTLC plates by resorcinol staining compared to the major gangliosides IV^{3}Neu5Ac-nLcOse$_4$Cer, IV^{6}Neu5Ac-nLcOse$_4$Cer, and VI^{3}Neu5Ac-nLcOse$_6$Cer, they showed high binding potential towards both viruses analysed in this study. This is in excellent agreement with data from Suzuki [7] who reported enhanced binding of influenza virus towards the sialyl Lewis^x ganglioside IV^{3}Neu5Ac,III^{3}Fuc-nLcOse$_4$Cer compared to other terminally sialylated but non-fucosylated monosialogangliosides.

The sialyl Lewis^x oligosaccharide is known as a ligand for the receptor family which binds to the cell surface sugar moieties of glycoconjugates, called the selectin family [30]. The sialyl Lewis^x structure has also been reported to be characteristic for some kind of human cancer cells [22]. However, VIM-2 carrying gangliosides from myeloid cells have recently been reported by Stroud et al. [31] as ligands for E-selectin expressing CHO-E cells. Furthermore, in a recent publication by Alon et al. [32] the general capability of gangliosides acting as ligands for selectins has been convincingly demonstrated. All these data imply that at least the E-selectin on endothelial cells and influenza A and Sendai virus hemagglutinins are capable of competing the sialyl Lewis^x and VIM-2 epitopes attached to lipid carriers on myeloid cells during inflammatory reactions.

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