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RECEPTOR GANGLIOSIDE CONTENT OF THREE HOSTS FOR SENDAI VIRUS

MDBK, HeLa, AND MDCK CELLS

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Specific gangliosides G_{D1a}, G_{T1b} and G_{Q1b} isolated from brain have been shown to function as receptors for Sendai virus by conferring susceptibility to infection when they are incorporated into receptor-deficient cells (Markwell, M.A.K., Svennerholm, L. and Paulson, J.C. (1981) Proc. Natl. Acad. Sci. USA 78, 5406–5410). The endogenous gangliosides of three commonly used hosts for Sendai virus: MDBK, HeLa, and MDCK cells were analyzed to determine the amount and type of receptor gangliosides present. In all three cell lines, G_{M3} was the major ganglioside component. The presence of G_{M1}, G_{D1a} and the more complex homologs of the gangliotetraose series was also established. In cell lines derived from normal tissue, MDBK and MDCK cells, gangliosides contributed 47–65% of the total sialic acid. In HeLa cells, gangliosides contributed substantially less (17% of the total sialic acid). The ganglioside content of each cell line was shown not to be immutable but instead to depend on the state of differentiation, passage number, and surface the cells were grown on. Thus, the ganglioside concentration of undifferentiated MDCK cells was found to be substantially greater than that of MDBK or HeLa cells, but decreased as the MDCK cells underwent differentiation. Changes in culture conditions that were shown to decrease the receptor ganglioside content of the cells resulted in a corresponding decrease in susceptibility to infection. The endogenous oligosialogangliosides present in susceptible host cells were shown to function as receptors for Sendai virus.

Introduction

As the first event of infection, paramyxoviruses such as Sendai virus recognize specific receptors on their host cell surface and through interaction with these receptors induce membrane fusion between the viral envelope and the surface membrane of their host cell. It has been shown that Sendai virus binds to specific oligosialogangliosides (G_{D1a} and its more complex homologs in the gangliotetraose series, G_{T1b} and G_{Q1b}) [1,2] isolated from brain and that these specific gangliosides confer susceptibility to infection when incorporated into receptor-deficient cells [2]. Moreover, G_{D1a}, has of itself the ability not only to bind the virus, but to bring it into close enough proximity to induce fusion when present in liposomes [3] or when inserted into receptor-deficient cells [2]. MDBK, HeLa, and MDCK cells are commonly used hosts for Sendai virus. Therefore, the ganglioside contents of these three host lines were analyzed to determine the amount and type of

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Abbreviations: ganglioside nomenclature according to Svennerholm [27]; MDBK cells, Madin-Darby bovine kidney cells; HeLa cells, Henrietta Lacks cells; MDCK cells, Madin-Darby canine kidney cells.

receptor gangliosides endogenously present which make these lines susceptible to infection.

Materials and Methods

Virus and cells

Sendai virus, Z strain (Parainfluenza 1 virus, hemagglutinating virus of Japan, HVJ) was propagated and purified as described [4]. To confirm the identity and purity of the virus, samples were iodinated before solubilization in SDS (surface-specific iodination) or after solubilization (nonvectorial iodination) using Iodo-gen [5] and the protein content analyzed by two-dimensional polyacrylamide electrophoresis [4]. The preparations were found to be more than 95% free of nonviral protein contaminants. Viral preparations were stored at −70 °C at a protein concentration of 5 mg/ml until the day of use.

MDBK and MDCK cells were grown in monolayer cultures in reinforced Eagle's medium containing 10% fetal bovine serum (heat-inactivated at 56 °C for 30 min). HeLa cells were grown in Dulbecco's minimum essential medium (containing 1 g glucose/l) and 10% fetal bovine serum. The three lines were checked for mycoplasm during this study and certified to be negative. Cells were used for experiments when 80–90% of the culture dish was covered by the cell monolayer ($2 \cdot 10^6$ cells per 35-cm dish). To determine cell identity by karyotyping, cells were grown in 25 cm^2 flasks. *N*-Deacetyl-*N*-methyl-colchicine (3 μg of Colcemid, Grand Island Biological Co.) was added to 5 ml of growth medium for 40 min to arrest the actively dividing cells in metaphase. The cells were harvested by trypsinization, fixed, and prepared for karyotyping by Giemsa banding as described [6]. 100 spreads were counted to establish the average chromosome number of each cell line.

Cells were infected by overlaying them with virus in Tris-saline (0.14 M NaCl/0.7 mM sodium phosphate/25 mM Tris-HCl (pH 7.2)) at a multiplicity of infection of three infectious particles per cell for 15 min at 37 °C. After three washes with Tris-saline, fresh growth medium was added to each dish. Virus production was quantitated by hemagglutination titer of the medium 48 h after inoculation, using formalinized chicken erythrocytes [7]. In cultures in which the infection was lytic, cell debris was removed by centrifugation for 5 min at $1000 \times g$ before titration.

Treatment of cells with sialidase, proteinases and exogenous gangliosides

Endogenous cell surface receptors were destroyed by incubation with 50 mU of *Vibrio cholerae* sialidase (EC 3.2.1.18, Calbiochem-Behring or Bethesda Research Laboratories proteinase-free neuraminidase, 1 IU/ml). MDBK cells were incubated with the sialidase for 2 h and HeLa cells for 4 h. MDCK cells were first treated with 0.5% trypsin for 15–20 min at 37 °C and then were centrifuged through a layer of 10% sucrose in Tris-saline. The MDCK cell pellet was then resuspended in 0.5 ml Tris-saline/bovine serum albumin (i.e., Tris-saline containing 1% bovine serum albumin) containing 50 mU sialidase and incubated for 60 min. Although different conditions were used for each cell line, the same end result was achieved, namely the production of receptor-deficient cells.

Individual ganglioside standards isolated from human brain [8] were dispersed in 0.5 ml of reinforced Eagle's medium or Dulbecco's minimum essential medium by vigorous vortexing for 2 min, then incubated with the receptor-deficient cells for 20 min at 37 °C. The oligosialoganglioside fractions prepared from the three host lines were incubated with receptor-deficient cells in a similar manner. Unadsorbed gangliosides were removed by washing the cells twice with Tris-saline/bovine serum albumin. The cells were then inoculated with Sendai virus as previously described.

To examine the effect of proteinases on virus-host cell interaction, cells were overlaid with freshly prepared, 0.5% trypsin (EC 3.4.21.4, Sigma, Type IX, 16260 BAEE units/mg protein) or 0.05% pronase (Calbiochem.-Behring 45 000 units/g dry wt.) in Tris-saline for 15–30 min at 37 °C, 10 min longer than that required to suspend them. This treatment was for the maximum time during which at least 95% of the cells maintained viability as evidenced by Trypan blue exclusion. Soybean trypsin inhibitor (1%) or bovine serum albumin (10%) was added to trypsin-treated or pronase-treated cells, respectively, and then they were centrifuged through a layer of 10% sucrose in

Tris-saline before adding the inoculating virus in medium containing 10% fetal bovine serum. After the 15 min inoculation period, unadsorbed virus was removed by centrifuging the cells through a layer of sucrose and the cells were replated in fresh growth medium and virus production measured at 48 h p.i. (postinoculation) as described.

Analysis of ganglioside content

Preparations of MDBK, HeLa, and MDCK cells were grown for analysis in 10-cm dishes, or roller bottles until 80–90% of the surface was covered by the monolayer, then harvested. In addition some preparations of MDCK cells were allowed to reach confluency and then cultured for 3 additional days until differentiation as evidenced by dome or blister formation was obvious. Ten roller bottles (the equivalent of one hundred 10-cm dishes) each of MDBK and MDCK cells and two hundred 10-cm plates of HeLa cells were used to establish the ganglioside pattern for each cell line. The quantitative ganglioside concentration and composition were then confirmed with smaller independent preparations of each cell line. A cell count and inoculation with Sendai virus was performed on each batch of cells to check the susceptibility of the cells to infection on the same day they were harvested for ganglioside analysis.

The cells were washed three times with ice-cold Tris-saline and then harvested by scraping them into additional Tris-saline. The scraped cells were centrifuged for 15 min at $500 \times g$ at 4°C and the pellet frozen at −20°C. Nanopure water was added to the cell pellet, 3 ml for smaller and 9 ml for larger cell preparations, and the suspension homogenized in a Potter-Elvehjem homogenizer at 4°C. A small aliquot of this homogenate was solubilized in 2% SDS and used for protein determination [9]. The lipids in the remainder of the sample were extracted by first adding methanol and then chloroform to the aqueous homogenate with continued homogenization to give a final solvent ratio of chloroform/methanol/water (4 : 8 : 3, v/v) [8]. After a 30-min extraction at room temperature, the sample was centrifuged for 10 min at $800 \times g$. The extraction procedure was repeated on the 8000 $g \cdot$ min pellet and the extracts were combined for lipid analyses. The delipidated pellet was saved for determination of protein-bound sialic acid [10].

The total lipid extract was desalted on a Sephadex G-25 column [11] and an aliquot of the extract was then used for assay of total phospholipids [12]. Gangliosides were separated from the neutral lipids by ion-exchange chromatography on Spherosil-DEAE-Dextran [13]. The gangliosides, all of which were retained on the resin, were eluted in two fractions. The one containing monosialogangliosides was eluted with 10 column volumes of 0.02 M potassium acetate in methanol and then those with two or more sialic acids (oligosialogangliosides) with 10 column volumes of 0.5 M potassium acetate in methanol plus 3 column volumes of 1.0 M potassium acetate in methanol.

The oligosialoganglioside fractions from MDBK, HeLa, and MDCK cells were then treated with 0.2 M KOH in methanol at 37°C overnight to hydrolyze any possible contaminating glycerophospholipids. These fractions were then neutralized with 1 M acetic acid. After dialysis for 48 h against several changes of water, the ganglioside fractions were freed from unesterified fatty acids, proteins, pigments, and other possible contaminants by chromatography on small columns of silica gel – approx. 1 g silica gel (Iatro-beads, Iatron Laboratories, Inc.) per 5 nmol sialic acid. The columns were first developed with 15 column volumes of chloroform/methanol/water (65 : 25 : 4, v/v) and then the oligosialogangliosides were eluted with 15 column volumes of chloroform/methanol/water (60 : 35 : 8, v/v).

The individual species of gangliosides in the mono- and oligosialoganglioside fractions were identified by comparing their migration on high-performance thin-layer plates developed in several solvents [10] before and after treatment with *V. cholerae* sialidase. The amount of each ganglioside was quantitated by densitometric scanning with a KM 3 scanner at 620 nm of ganglioside bands visualized with resorcinol. The ganglioside G_{M1} which is the end-product after sialidase treatment of all gangliosides of the ganglio-tetraose series was also identified and quantitatively determined by its binding to cholera toxin. The gangliosides which migrated as G_{M3} and G_{D3} were identified by hydrolyzing them with sialidase and the neutral dihexoside formed was isolated by TLC developed in chloroform/methanol/water (65 : 25 : 4, v/v) and shown to be lactosylceramide.

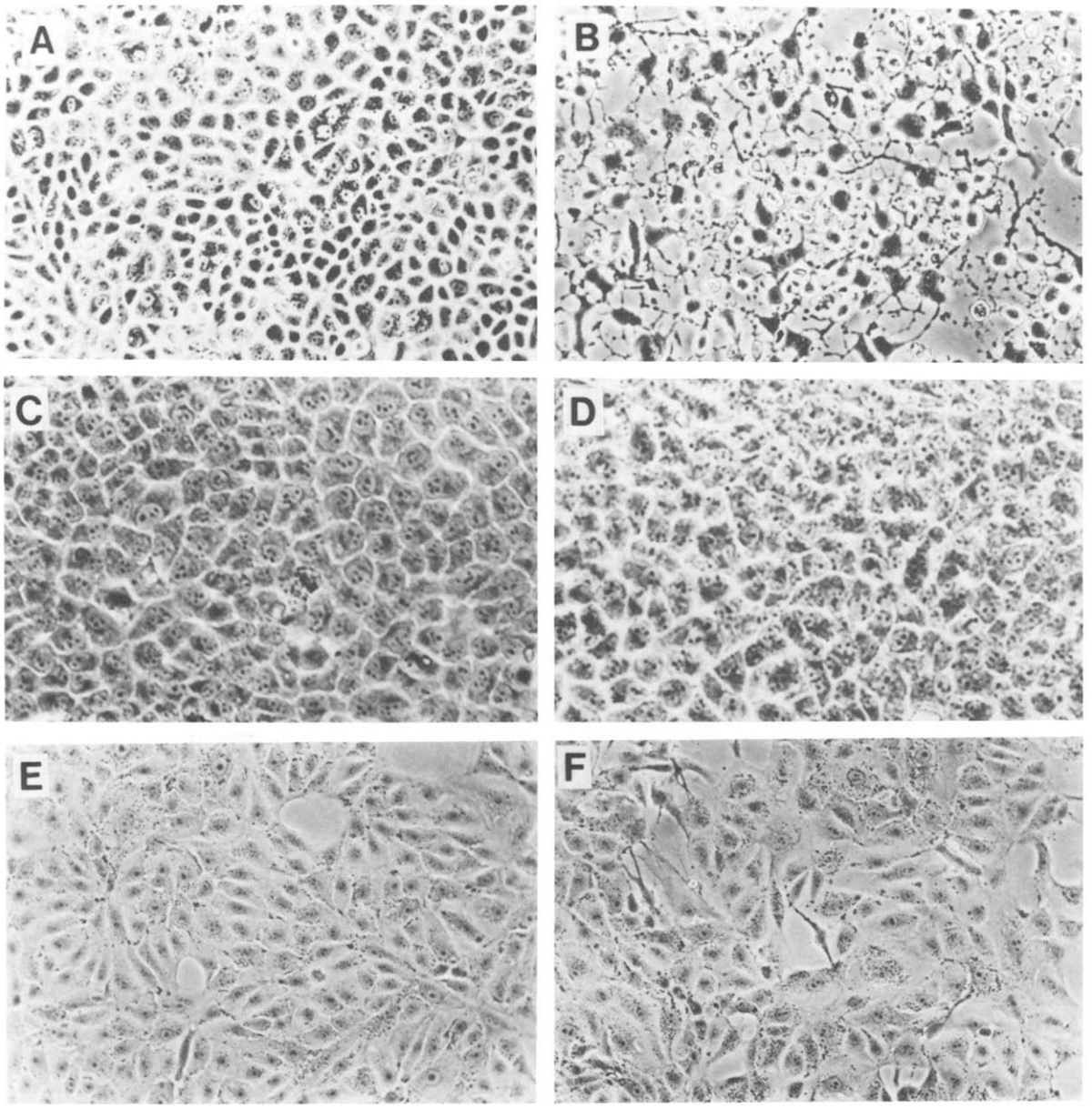

Fig. 1. Response of three different hosts to infection by Sendai virus. MDBK, HeLa, and MDCK cells are shown in their native state in A, C and E, respectively. The changes in morphology due to infection are apparent by 24 h p.i. and vary in degree from the lytic response of MDBK cells (B) to partial lysis of the MDCK cells in monolayer (F) to an essentially nonlytic response by HeLa cells (D).

Results

Characterization of infection

The identity of MDBK, HeLa, and MDCK cells was confirmed by karyotyping. All produced similar amounts of progeny virus (256–512 HAU */ml) by 48 h (p.i.) but showed considerable variation in their response to infection (Fig. 1). The infection of MDBK cells by Sendai virus, Z strain, is lytic. The first evidence of virus bud-

* HAU, hemagglutination unit.

TABLE I

EFFECT OF PROTEINASES AND SIALIDASE ON THE CELL-SURFACE RECEPTORS FOR SENDAI VIRUS

Monolayers of MDBK, HeLa, and MDCK cells were treated with 0.5% trypsin or 0.05% pronase for 15–30 min or with 5 mU of *V. cholerae* sialidase for 1–4 h. Proteinase inhibitors or bovine serum albumin were added to the proteinase-treated cells before inoculation with Sendai virus. Virus production was measured by hemagglutination titer at 48 h p.i.

Cell	Virus production (HAU/ml) in:		
	MDBK cells	HeLa cells	MDCK cells
Native	256	256	256
Proteinase-treated	256	512	256
Sialidase-treated	< 2	< 2	< 2

ding from the cell surface was the marked cytopathic effect observed at 22–24 h p.i. as the appearance of spindle-shaped cells with long and irregular cytoplasmic processes (Fig. 1B). This effect was less evident in MDCK (Fig. 1D) and almost undetectable in HeLa cells (Fig. 1F) at the end of the same time period.

Production of receptor-deficient cells and conferral of infectivity by incorporation of specific gangliosides

Our previous studies have shown that incubation of MDBK cells with sialidase produces receptor-deficient cells by destroying their endogenous receptors for Sendai virus [14]. This study extends this observation to two additional hosts (Table I). Sialidase treatment of MDBK, HeLa, and MDCK cells for 2–4 h made them resistant to infection (less than 2 HAU/ml). Proteinase treatment of native MDBK and MDCK cells had no effect on their interaction with Sendai virus, but the same treatment of HeLa cells made them significantly more receptive to infection by Sendai virus (Table I, 256 vs. 512 HAU/ml). Trypsin treatment released approx. 70% of the total sialic acid from HeLa cells.

Individual, homogeneous ganglioside standards of G_{M1}, G_{D1a}, and G_{Q1b} were incubated with the receptor-deficient cells (Table II). In good agreement with previous binding studies [1,2], G_{D1a} and G_{Q1b} demonstrated receptor function but G_{M1} did not. In all three cell lines, G_{Q1b} was effective as a receptor at a 100-fold lower concentration than G_{D1a}. To achieve susceptibility to infection, the incubation of receptor-deficient cells with G_{D1a} or G_{Q1b} had to be done in protein-free medium (reinforced Eagle's medium or Dulbecco's minimum essential medium without serum) and at 37°C. Incubation of gangliosides with the cells in medium containing 10% serum or 1% bovine serum albumin, or at 4°C showed no evidence of restoration of infectivity (less than 2 HAU/ml).

TABLE II

CONFERRAL OF SUSCEPTIBILITY TO INFECTION TO RECEPTOR-DEFICIENT CELLS BY INCORPORATION OF SPECIFIC GANGLIOSIDES

MDBK, HeLa, or MDCK cells were treated with 50 mU of sialidase for 2–4 h and then incubated for 20 min with purified brain gangliosides G_{M1}, G_{D1a} or G_{Q1b} in protein-free medium. Virus production was measured by hemagglutination titer of the medium at 48 h p.i.

Cells	Addition	Virus production (HAU/ml) in:		
		MDBK cells	HeLa cells	MDCK cells
Native	None	256	256	256
Sialidase-treated	None	< 2	< 2	< 2
Sialidase-treated	1 nmol G_{M1}	< 2	< 2	< 2
Sialidase-treated	1 nmol G_{D1a}	64	256	256
Sialidase-treated	0.1 nmol G_{D1a}	4	64	64
Sialidase-treated	0.01 nmol G_{D1a}	< 2	16	8
Sialidase-treated	0.1 nmol G_{Q1b}	128	512	256
Sialidase-treated	0.01 nmol G_{Q1b}	64	256	128
Sialidase-treated	0.001 nmol G_{Q1b}	4	32	32

A direct comparison of the maximum HAU/ml produced by MDBK cells after the addition of exogenous gangliosides with that of native cells is somewhat misleading because of a cell loss of about 50% of that line during the succession of treatment and accompanying washes. So for MDBK cells, virus production achieved by incubation with gangliosides never exceeded 128 HAU/ml. There was not a problem of substantial cell loss with MDCK and HeLa cells. For HeLa cells, the state of susceptibility achieved by incubation with the exogenous ganglioside G_{Q1b} was actually greater than that of native cells (512 vs. 256). The addition of 0.1 nmol G_{Q1b} for up to 3 h or 1 nmol of G_{D1a} for 24 h to native HeLa cells did not increase virus production above the 256 HAU/ml level of control, infected cells.

The membrane components of MDBK, HeLa and MDCK cells: their changes with state of differentiation, passage number, and substratum

One of the three hosts used in this study, MDCK cells, are known to undergo spontaneous differentiation in culture as evidenced by the formation of fluid-filled, multicellular hemicyst (domes or blisters) indicative of the vectorial fluid transport of kidney tissue [15]. However, differentiated cells were found to be equally as susceptible to infection as undifferentiated cells (256 HAU/ml/$2 \cdot 10^6$ cells for both states). For analyses of their membrane components, several batches of MDBK, HeLa, undifferentiated MCDK cells and differentiated MDCK cells were grown and processed as described in Materials and Methods. In general, undifferentiated MDCK cells had a higher concentration of phospholipids, sialoglycoproteins, and gangliosides than any of the other types examined when compared on a per mg of protein basis (Table III) or on a per cell basis. The concentration of these membrane components decreased 2- to 3-fold as MDCK cells underwent differentiation. The distribution of sialic acid between lipid and protein fractions from the three cell lines was also noteworthy. In cells derived from normal tissue, i.e., MDBK and MDCK cells, gangliosides contributed 47–64% of the sialic acid found in these cell types. In HeLa cells which are derived from a cervical carcinoma, gangliosides constituted a considerably smaller proportion (17%) of the total sialoglycoconjugates.

Although the general membrane composition of the cells remained relatively constant from batch to batch of cells for each type (Table III), the ganglioside composition showed considerable variation. This was demonstrated by the comparison of two different passages of MDCK cells (Table IV). G_{M3} was always the most predominant ganglioside but its relative proportion to the

TABLE III

ANALYSIS OF MEMBRANE COMPONENTS OF MDBK, HeLa, AND MDCK CELLS

The membrane composition of three epithelial cell lines were compared. On the day the cells were harvested for analysis, susceptibility to Sendai virus infection by each cell line was confirmed. Two separate preparations of MDBK, HeLa, and differentiated MDCK cells, and three preparations of undifferentiated MDCK cells were analyzed.

	Cell type			
	MDBK	HeLa	Undifferentiated MDCK	Differentiated MDCK
Cell density ($\times 10^{-7}$ cells/10-cm dish)	0.94– 1.1	0.68– 0.76	0.52– 0.72	2.5– 2.5
Contents (nmol/mg protein)				
Phospholipids	353 –374	340 –402	490 –570	270 –340
Protein-bound sialic acid	6.8 – 7.1	12.2 – 14.0	11.7 – 21.9	6.6– 10.4
Lipid-bound sialic acid (gangliosides)	7.6 – 7.7	2.5 – 2.9	21.4 – 31.1	5.9– 9.4
% Total sialic acid in gangliosides	52 – 53	17 –17	39 – 65	47 – 47

TABLE IV

VARIATION IN GANGLIOSIDE COMPOSITION WITH PASSAGE NUMBER

Two preparations of undifferentiated MDCK cells were harvested from different passages of cells. Differentiated MDCK cells were prepared from the same passage of cells as the second batch of undifferentiated cells. tr, trace.

	% of sialic acid in total gangliosides		
	Undifferentiated MDCK-1	Undifferentiated MDCK-2	Differentiated MDCK-2
G_{M3}	93	77	71
L_{M1}	–	1	1
G_{M1}	3	5	5
G_{D3}	1	5	4
G_{D1a}	2	7	10
G_{D1b}	1	4	5
G_{T1b}	1	2	4
G_{Q1b}	tr	tr	tr

oligosialogangliosides varied depending on which passage of cells was examined. The preparations of undifferentiated MDCK cells (MDCK-1 and MDCK-2) from two different passages of cells differed substantially in composition.

That the differences observed in ganglioside composition were inherent in the cells themselves rather than an artifact arising during the extraction and purification of the gangliosides is supported by two pieces of evidence. First, cells from the same passage had similar compositions when processed as two independent samples. Even though the state of differentiation is different, undifferentiated and differentiated MDCK cells from the same passage (MDCK-2 of Table IV) have almost indentical ganglioside compositions. Second, when MDBK and MDCK cells were grown on a different substratum, glass instead of plastic, the relative proportion of the oligosialogangliosides dropped to less than 3% of the total gangliosides present and this change was reflected by a 16-fold decrease in the susceptibility of the cells to infection. When cells were replated on plastic, they regained full susceptibility to infection within 24 h.

Endogenous receptor ganglioside content of MDBK, HeLa and MDCK cells

The endogenous ganglioside composition of large preparations (100–200 plates) of MDBK, HeLa, and MDCK cells were extensively analyzed. For each cell type, the gangliosides were tentatively identified by their migration on thin-layer chromatography relative to reference gangliosides from human brain (Fig. 2). Coincidence of migration plus analysis, as described in Materials and Methods, allowed positive identification of the major bands of the cell gangliosides as belonging to G_{M3}, G_{D3}, G_{M1}, G_{D1a} and its higher homologs in the gangliotetraose series of gangliosides (Table V). The ganglioside which migrated slightly slower than authentic G_{M2} was tentatively identified as L_{M1}. In all three host cell lines, G_{D1a}, the simplest ganglioside known to function as a receptor for the virus, was present as well as its more complex homologs G_{T1b} and G_{Q1b}, all of which have been shown to have receptor activity [2]. Although G_{Q1b} was present in only trace amounts its high affinity for the virus makes it effective as a receptor at a

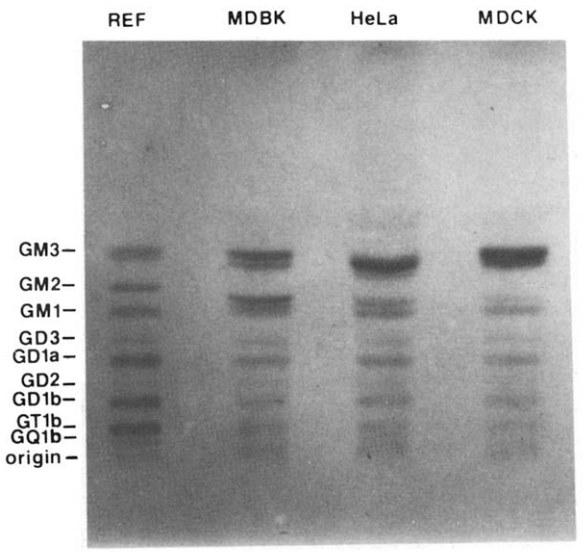

Fig. 2. Comparison of ganglioside composition of MDBK, HeLa, and MDCK cells. Total ganglioside extracts from subconfluent MDBK, HeLa, and MDCK cells were prepared by pooling the mono- and oligosialoganglioside fractions described in Materials and Methods. Aliquots of each extract were applied to HPTLC plates (Merck, Silica gel 60) which were developed in chloroform/methanol/0.25% KCl (aqueous) (50:40:10, v/v). The reference gangliosides were prepared from an adult human brain with the addition of G_{M2} and G_{M3}. Gangliosides were visualized by spraying with resorcinol reagent.

TABLE V

GANGLIOSIDE CONTENT OF MDBK, HeLa AND MDCK CELLS

Total ganglioside content (lipid-bound sialic acid) was quantitated using the resorcinol assay. Individual gangliosides were identified by migration on thin-layer plates (Fig. 2) and structural analyses. Values are expressed as nmol sialic acid/mg protein. tr, trace.

	nmol of sialic acid/mg protein			
	MDBK cells	HeLa cells	Undifferentiated MDCK cells	Differentiated MDCK cells
Gangliosides	7.7	2.5	31.1	9.4
G_{M3}	4.0	1.6	24.1	6.7
L_{M1}	1.5	0.2	0.3	0.1
G_{M1}	1.3	0.2	1.4	0.5
G_{D3}	0.2	0.1	1.4	0.4
G_{D1a}	0.3	0.2	2.3	0.9
G_{D1b}	0.2	0.2	1.1	0.5
G_{T1b}	0.4	0.1	0.6	0.4
G_{Q1b}	tr	tr	tr	tr

TABLE VI

THE FUNCTIONING OF ENDOGENOUS MDBK, HeLa, AND MDCK CELL GANGLIOSIDES AS SENDAI VIRUS RECEPTORS

Oligosialoganglioside fractions were prepared from MDBK, HeLa, and MDCK cells. An aliquot of each fraction containing 0.3 nmol G_{D1a} plus G_{T1b} was added to receptor-deficient HeLa cells for 20 min. After removal of the unadsorbed ganglioside, the cells were inoculated with virus and 48 h later assayed for virus production by hemagglutination titer. Standards of G_{D1a} and G_{M1} isolated from human brain were used at a concentration of 0.3 nmol for comparison of receptor function.

Addition	Virus production (HAU/ml)
None	< 2
G_{M1}	< 2
G_{D1a}	256
Oligosialogangliosides	
From MDBK cells	256–512
From HeLa cells	256–512
From undifferentiated MDCK cells	256
From differentiated MDCK cells	256

100-fold lower concentration than G_{D1a} or G_{T1b} ([2], Table II of this study), and so even trace amounts of it are significant in terms of susceptibility to infection.

The oligosialoganglioside fractions from MDBK, HeLa, and MDCK cells (Table V) contained enough G_{D1a} by itself to fully account for the susceptibility to infection these cells displayed. As further confirmation of the presence of endogenous receptor gangliosides, these fractions were tested for functional receptor activity by adding aliquots of them to receptor-deficient HeLa cells. For each fraction, it was assumed that G_{D1a} and G_{T1b} had equivalent receptor activity [2], so an aliquot containing a total of 0.3 nmol of these along with the other oligosialogangliosides was incubated with the other oligosialogangliosides was incubated with the receptor-deficient cells. Standards of G_{M1} and G_{D1a} were used to calibrate the system. In all cases, the incorporation of the gangliosides endogenously present in MDBK, HeLa and MDCK cells produced full susceptibility to infection (Table VI). The higher HAU value obtained with the cell oligosialogangliosides than with standard G_{D1a} is reminiscent of the results with HeLa cells in Table II and may be due to the occurrence of trace amounts of G_{Q1b} in the cell samples.

Discussion

The oligosialogangliosides endogenously present in three typical host cell lines have been shown in this study to function as receptors for Sendai virus. These results complete the definition of a receptor * for the first mammalian virus. The present study, which relies on the actual biological event of infection to functionally identify receptors, confirms the suggestions of previous studies which employed model systems. In these systems, brain gangliosides were shown to specifically bind the virus when they were incorporated into liposomes [16] or adsorbed onto plastic [1]. Moreover, when these putative receptors were incorporated into receptor-deficient cells, they conferred susceptibility to infection [2]. The final piece of evi-

* A virus receptor is a macromolecule or complex of macromolecules naturally occurring on the host cell surface which specifically binds the virus and through this binding facilitates the subsequent events of infection.

dence needed to designate the ganglioside G_{D1a} and its higher homologs as receptors was to demonstrate their actual presence in host cells. In each of the hosts examined in this study, receptor gangliosides were shown to be present when the cells were susceptible to infection.

That host cell receptors for Sendai virus might be lipid in nature was first suggested in 1974 [16] when it became apparent that for this virus infectious particles were those which caused membrane fusion between the viral envelope and host cell membrane [17,18]. For this membrane fusion to occur, it is estimated that the two membranes must approach to within 10–15 Å of each other [19], a situation favoring attachment to the proximal oligosaccharides of glycolipids over the more distal ones of proteins. A recent study employing the liposome system [3] has demonstrated that G_{D1a} causes specific binding of the virus and induces fusion in the absence of protein, indicating that G_{D1a} has inherent in its structure total receptor function.

Although gangliosides have full receptor function in the absence of protein, proteins on the cell surface may influence the expression of gangliosides as receptors. Evidence for a modulating effect of proteins was observed in this study (Table I). In HeLa cells, although analytical results documented that enough receptor gangliosides were present for full susceptibility to infection, stripping the cell surface of some protein by the action of proteinases revealed these receptors more fully and caused the cells to become more susceptible than in their native state. Similar results have been observed under two other sets of conditions: (1) during the first minutes of sialidase treatment of MDBK cells (Fig. 1 of Ref. 14), and (2) upon addition of receptor gangliosides to HeLa cells which have undergone extensive sialidase treatment (Table II, this study). In all three cases, an environment was created in which the receptor gangliosides existed with less than the usual amount of nonreceptor sialoglycoconjugates. A number of investigators have suggested that glycosphingolipids may be masked by their association with other membrane components (see Ref. 20 for recent review). Thus, the susceptibility of a potential host to infection by Sendai virus may be determined not only by the presence or absence of appropriate receptors such as G_{D1a} but also by their accessibility to the virus.

The second point emphasized by the results of this study is that the ganglioside content of any particular cell line is not immutable. Two of the factors contributing to this variability, state of differentiation and growth substratum, have been defined by this study. The spontaneous differentiation of MDCK cells in culture is accompanied not only by morphological changes such as dome formation but also by a substantial decrease in sialoglyconjugates (Table III). Similar changes have been noted in comparing neuroblastoma cells at different levels of differentiation [21] or cell density [22]. Another defined factor influencing ganglioside content is the surface the cells are grown on. Substitution of glass roller bottles for plastic ones resulted in a dramatic drop in the concentration of the more complex gangliosides. Similar results have been noted by others. Two recent reports demonstrated that the carbohydrates of glycoproteins vary dramatically depending on the substratum the cells were grown on [23,24], but the ganglioside content of these cells were not examined in these studies.

In addition to the defined factors of state of differentiation and substratum, other factors as yet undefined gave rise to variation in ganglioside content between different passages of cells. This variation observed within a period of 2 months within a single line grown in the same laboratory helps to explain why the same line, such as HeLa cells, when grown in different laboratories may have varying compositions [25,26].

Because the ganglioside content can change in response to several factors, it is imperative before designating cells as host cells to establish their susceptibility to infection on the same day as they are harvested for analysis. By including this essential control, we were able to detect the 16-fold increase in susceptibility which occurred when the MDCK cells grown on glass were transferred to a plastic substratum and acquired additional oligosialogangliosides. In every case examined in this study, susceptibility to infection by Sendai virus correlated with the presence of oligosialogangliosides of the gangliotetraose series.

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