Identification of the Sialic Acid Structures Recognized by Minute Virus of Mice and the Role of Binding Affinity in Virulence Adaptation*

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Sialic acid binding is required for infectious cell surface receptor recognition by parvovirus minute virus of mice (MVM). We have utilized a glycan array consisting of ~180 different carbohydrate structures to identify the specific sialosides recognized by the prototype (MVMp) and immunosuppressive (MVMi) strains of MVM plus three virulent mutants of MVMp, MVMp-K368R, and MVMp-K368R. All of the MVM capsids specifically bound to three structures with a terminal sialic acid-linked α2–3 to a common Galβ1–4GlcNAc motif: Neu5Aca2–3Galβ1–4GlcNAc (3’SiaLN-LN), Neu5Aca2–3Galβ1–4GlcNAc (3’SiaLN-LN), and Neu5Aca2–3Galβ1–4(Fucα1–3)-GlcNAcβ1–3Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4(Fucα1–3)Galβ1–4GlcNAc (SLeα–Leα–Leα–)2. In addition, MVMi also recognized four multisialylated glycans with terminal α2–8 linkages: Neu5Aca2–8Neu5Aca2–8Neu5Aca2 ((Sia)2), Neu5Aca2–8Neu5Aca2–8Neu5Aca2–8Neu5Aca2–8Neu5Aca2–8Neu5Aca2–8Neu5Aca2–3Galβ1–4Glc (GD3), Neu5Aca2–8Neu5Aca2–8Neu5Aca2–8Neu5Aca2–8Neu5Aca2–3Galβ1–4Glc (GT3), and Neu5Aca2–8Neu5Aca2–8Neu5Aca2–3Galβ1–4Glc (GD2). Interestingly, the virulent MVMp-K368R mutant also recognized GT3. Analysis of the relative binding affinities using a surface plasmon resonance biospecific interaction (BLAcore) assay showed the wild-type MVMp and MVMi capsids bind with higher affinity to selected glycans compared with the virulent MVM mutants. The reduced affinity of the virulent MVM mutants are consistent with previous in vitro cell binding assays that had shown weaker binding to permissive cells compared with wild-type MVMp. This study identifies the sialic acid structures recognized by MVM. It also provides rationale for the tropism of MVM for malignant transformed cells that contain SLcα motifs and the neurotropism of MVMi, which is likely mediated via interactions with multisialylated glycans known to be tumor cell markers. Finally, the observations further implicate a decreased binding affinity for sialic acid in the in vivo adaptation of MVMp to a virulent phenotype.

Attachment to a cell surface receptor is an essential first step in the life cycle of many viruses. The initial viral attachment frequently determines the tissue tropism, and subsequent interactions determine the pathogenic outcome. In many virus-host interactions, glycan chains often function as initial recognition molecules, with or without a protein component. Glycans are a major element of living systems, with >50% of all proteins being glycosylated. They are abundantly present in the outer cell surface and thus serve as convenient targets for viral attachment. The recognition of specific glycan motifs by viruses can be mediated by a specific viral surface protein component, such as the hemagglutinin of influenza virus (1), or specific regions of the viral capsid itself, as is proposed for members of the single-stranded DNA Parvoviridae family (2–8). The initial attachment of viruses to host cells is followed by an internalization process that often involves concerted actions of many factors, such as co-receptors and/or the endocytotic machinery.

Two strains of minute virus of mice (MVM), an autonomous member of the Parvoviridae family, have served as models for studies aimed at identifying parvovirus capsid regions involved in cellular host range (tropism) and pathogenicity determination (9–11). The prototype strain of MVM (MVMp) possesses a fibrotropic host cell preference, and infection is asymptomatic, whereas the immunosuppressive strain (MVMi) infects hematopoietic and lymphotropic cells and can cause a lethal infection in certain genetically inbred newborn mice and adult mice with the severe combined immunodeficient (SCID) phenotype (12–16). Previous studies show that pretreatment of susceptible cells with neuraminidase or proteinase K inhibits MVM infection indicating that sialic acid attachment, in the context of a glycoprotein, is an essential step in its cellular recognition and tropism (11, 17). The protein component of the infectious receptor is unknown.

MVM has a single-stranded DNA genome of ~5 kb packaged within capsids that are ~260 Å in diameter. The genomic DNA encodes non-structural and structural (capsid) proteins, and the MVM capsid is composed of 60 copies (total) of overlapping capsid viral proteins (VPs), VP1, VP2, and VP3 (in DNA con-
binding assays with virulent MVMp viruses in which two of the
that their mode of pathogenicity is different (24).
acid position 368. The virulent MVMp mutants retain the
changing the MVMp residue to that found in MVMi at amino
7–13 days post-infection before death is prolonged, occurring
20–60 days post-infection compared with ~7–13 days post-
infection for MVMi, and there is no hematopoeisis, suggesting
their mode of pathogenicity is different (24). In vitro cell
binding assays with virulent MVMp viruses in which two of the
mutant residues were re-engineered into the original wt virus,
either alone (MVMp-I362S or MVMp-K368R) or in combina-
tion (MVMp-I362S/K368R), showed reduced binding to a nat-
urally abundant receptor on fibroblast cells compared with wt
MVMp (11). In the same study, pretreatment of the fibroblast
cells with neuraminidase inhibited binding by the wt MVMp
and mutant viruses (11), confirming previous reports identify-
ing sialic acid as the carbohydrate component required for cel-
ular recognition (17). Residues 362 and 368 are immediately
proximal to the pocket recently identified as a sialic acid bind-
ing region (Fig. 1) at the icosahedral 2-fold axis of the wt MVMp
capsid (11). Thus, the altered cell binding properties of the
mutants suggested that the acquired virulence phenotype was
mediated through a modified interaction with a sialylated cell
surface receptor and that avidity or affinity for the receptor is
likely a determinant of virulence (11).

In this study, our goal was to identify the specific sialic acid
motifs recognized by MVMi and MVMp and three virulent
MVMp mutants, MVMp-I362S, MVMp-K368R, and MVMp-
I362S/K368R, using a glycan array containing ~180 carbo-
hydrate motifs. The sugars in the array, developed by the Consor-
tium for Functional Glycomics, represent the major glycan
structures found in glycoproteins and glycolipids.3 This report
represents the first use of the array to study the specific inter-
action between whole intact virus particles and the immobi-
lized glycans. All of the MVM viruses recognized the same
α2–3 sialylated glycans linked to a Gal-GlcNAc motif, with
MVMi and the MVMp-K368R mutant showing expanded rec-
ognition to α2–8 multisialylated glycans. The specificity of the
interaction(s) between the glycans was identified and the MVM
viruses were confirmed by secondary analysis using a surface
plasmon resonance (SPR) biospecific interaction (BIAcore)
assay. This technique also measured the relative binding affini-
ity of MVMi, MVMp, and the virulent MVMp mutants. Our
observations point to an infectious mechanism facilitated by
recognition of specific sialic acid structures on the cell surface
by MVM and further implicate a mechanism involving differ-

3 The compound library was produced by the Consortium for Functional Gly-
comics, functionalglycomics.org.
ent receptor affinity as a determinant of in vivo parvovirus virulence.

**EXPERIMENTAL PROCEDURES**

**Virus Production**—Baculovirus constructs expressing the VP2 of wt MVMi and MVMp and the virulent MVMp mutants (MVMp-I362S, MVMp-K368R, and MVMp-I362S/K368R) that self-assemble into VLPs were generated as previously described (11). Briefly, VLPs (devoid of genome) were produced in S9 insect cells, released by freezing and thawing, and then purified through a series of centrifugal sedimentation and density equilibrium gradients as previously reported (21). The concentrations of the viruses were adjusted to 1–3 mg/ml for SDS-PAGE and electron microscopy to check the purity and capsid integrity. For electron microscopy experiments, 4 μl of purified virus was spotted onto a 400-mesh carbon-coated copper grid (Ted Pella, Inc., Redding, CA) for 1 min before blotting with filter paper (Whatman glycain 5). The samples were then negatively stained with 4 μl of 2% uranyl acetate for 15 s, blotted dry, and viewed on a Joel JEM-100CX II electron microscope.

**Glycan Array Screening of MVM Viruses**—Two cores of the Consortium for Functional Glycomics (a National Institutes of Health, National Institute of General Medical Sciences initiative), cores D and H, have developed a high throughput glycan screening assay for identifying specific carbohydrate binding partners for proteins using a streptavidin/biotin glycan array. The screening system utilized for the current study contained ~180 different glycans immobilized as biotinylated glycosides on a 384-well streptavidin-coated plate. The glycoside probes are listed on the Consortium for Functional Glycomics web site as Glycan Array Version 3 at functionalglycomics.org/static/consortium/resources/resourcecoreh5.shtml. The MVM virus samples MVMi, MVMp, MVMp-I362S, MVMp-K368R, and MVMp-I362S/K368R were screened in binding buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, 0.05% Tween 20, 1% bovine serum albumin) as previously described (25). Briefly, biotinylated glycosides (26) were bound to streptavidin-coated microtiter plates in replicates of n = 4. Precoated plates were washed three times with 100 μl of wash buffer (binding buffer minus bovine serum albumin) prior to incubation. A stock solution of each virus preparation (30 μg/ml) was added to each well and incubated at room temperature for 1 h. The plates were washed and incubated with a rabbit anti-MVM capsid antibody (diluted 1:5,700–15,000). The plates were washed and incubated with goat anti-rabbit IgG-Alexa488 (Molecular Probes) at 5 μg/ml. All incubations were for 1 h at room temperature. The plates were washed and read in 25 μl of wash buffer on a Victor-2TM 1420 Multilabel Counter (PerkinElmer Life Sciences) at excitation 485 nm/emission 535 nm. To analyze the results, all glycans were ranked according to their signal-to-noise (S/N) ratio by dividing their mean relative fluorescence units (from the four replicates) by the mean background generated in the control wells lacking glycodies. This value was compared with the average S/N for all wells in the array, and the results were then ranked as high affinity (>3 times the average S/N), medium affinity (>2 times the average S/N), and low affinity (>1 times the average S/N).

**Spr BIAcore Assay to Measure Binding Specificity and Relative Affinity of MVM-sialylated Glycan Interactions**—Secondary analysis with SPR BIAcore has been used to confirm the specificity of protein interaction with glycans bound in glycan arrays (see Ref. 25). We used a similar strategy to analyze the binding of the MVM viruses to the glycans identified in the array. Three different representative glycans, Neu5Aca2–3Galβ1–4GlcNAcβ1–4Galβ1–4GlcNAc (3’SiaLN-LN, glycan 106), Neu5Aca2–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAc (3’SiaLN-LN-LN, glycan 194), and Neu5Aca2–8Neu5Aca2–8Neu5Aca2–3Galβ1–4Glc (GT3, glycan 111), recognized by the MVM viruses were used for these assays. All SPR experiments were performed at 25°C on a BIAcore 3000 (B3000) instrument. The running buffer was 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM MgCl₂, and 2 mM CaCl₂ containing 0.005% p20. Biotinylated oligosaccharides were captured on a research grade streptavidin-coated sensor chip. The chip was pretreated with three injections of 1 mM NaCl/50 mM NaOH at 5 μl/min for 1 min/injection. A 10-fmol/μl solution of each biotinylated oligosaccharide was then injected at 2 μl/min for varying lengths of time until optimal amounts of the glycan were captured on each independent surface. The selected glycans 106, 194, and 111 were studied using one streptavidin sensor chip. A control (non-binding, non-sialylated) glycan, Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAc (LN-LN-LN-LN, glycan 35), was also captured on the sensor chip. The specific binding of the MVM viruses to the test glycans were measured using the in-line reference subtraction feature of the B3000. Increasing concentrations of the MVM viruses MVMi, MVMp, MVMp-I362S, MVMp-K368R, and MVMp-I362S/K368R were injected at a flow rate of 40 μl/min over all four surfaces of the sensor chip. Bound capsids were removed by injecting a 10 mM solution of glycine at pH 2.0. Binding curves for the interaction of the MVM viruses with the three different glycans were compared by overlaying the sensorgrams obtained on each surface.

**RESULTS**

To identify the specific sialic acid structures recognized by MVM and biophysically evaluate the individual role of the two virulent MVMp VP mutations I362S and K368R on receptor affinity, recombinant baculoviruses expressing the VP2 of MVMi, MVMp, and the three virulent MVMp mutants were used to produce VLPs for glycan array screening and SPR Bla-core assays. The VLP samples produced were purified to high homogeneity and observed to be intact prior to use (Fig. 2).

Of the ~40 sialylated glycans in the array, all of the MVM viruses specifically bound to only three structures with terminal α2–3-linked sialic acid, 3’SiaLN-LN, 3’SiaLN-LN-LN, and Neu5Aca2–3Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4(Fucα1–3)GlcNAc (sLe³–Le³–Le³) (Fig. 3). However, MVMi binding was extended to four additional multisialylated glycans with α2–8 linkages, Neu5Aca2–8Neu5Aca2–8Neu5Aca2 (Sia)₃, Neu5Aca2–8Neu5Aca2–8Neu5Aca2–3Galβ1–4Glc (GD3), GT3, and Neu5Aca2–8Neu5Aca2–8Neu5Aca2–3GalNAcβ1–4Galβ1–4Glc (GD2) (Fig. 3). These four glycans comprise all of the multisialylated glycans
with α2–8 linkage in the array. The GT3 glycan was also recognized by the virulent MVMp-K368R mutant that contains the MVMi amino acid type at position 368 (Fig. 3). All of these glycans were ranked as having high affinity. For the three glycans recognized by all of the MVM viruses, the glycan bound the tightest, as ranked by relative fluorescence, was 3'SiaLN-LN-LN (glycan 194), followed by sLeα-Leα-Leα (glycan 195), and then 3'SiaLN-LN (glycan 106) (Fig. 3). For the additional glycans recognized by MVMi, the binding ranking order was GT3 (glycan 111) > GD2 (glycan 110) > GD3 (glycan 43) > (Sia)α (glycan 156). The affinity of MVMi for GT3 and GD2 was comparable with its affinity for 3'SiaLN-LN-LN and sLeα-Leα-Leα, and that for GD3 is ~10% less (Fig. 3). The recognition of GT3 by the MVMp-K368R mutant was at ~10% of its binding to 3'SiaLN-LN-LN (Fig. 3).

Significantly, the MVMp, MVMp-I362, and MVMp-I362S/K368R viruses only recognized sialosides with NeuAc2–3 linked to Gal-GlcNAc (LN), with glycan 195 containing branched fucose residues. A motif that was similar to glycan 106 but with NeuAc2–6-linked sialic acid (glycan 109) was not recognized. This motif is also very common in glycoproteins and glycolipids. In addition, the length of the oligosaccharide linked to the terminal NeuAc2–3 also appeared to be critical for recognition by these viruses, with a pentasaccharide being the minimal length of the glycans bound (Fig. 3). There was no specificity for the shorter NeuAcα2–3Galβ1–4GlcNAcβ (3'SiaLN, glycan 38), and the affinity for the longer chain glycan heptasaccharide glycan 194 was higher than for the pentasaccharide glycan 106 (Fig. 3).

Three glycans, 3'SiaLN-LN-LN (glycan 194), 3'SiaLN-LN (glycan 106), and GT3 (glycan 111), were used in SPR BIACore experiments to validate the results of the glycan array and to determine the relative binding affinity of the MVM viruses for these sialylated molecules. A non-sialylated glycan containing the LN motif that was not recognized by the MVM viruses, LN-LN-LN (glycan 35), was captured on the fourth surface of a sensor chip and used as a negative control. Although it is assumed that there could be 60 receptor binding sites on the T = 1 MVM capsid, the exact stoichiometry of the MVM-sialic acid interaction is not known, thus the binding data were not analyzed for quantitative kinetic information. Instead, a relative binding affinity was assessed from the data based on visual examination of the binding curves.

The binding curves for MVMp with different concentrations of 3'SiaLN-LN-LN (glycan 194) and 3'SiaLN-LN (glycan 106) are shown in Fig. 4. A concentration-dependent increase in SPR response was observed consistent with specific recognition of the captured glycans. Similar concentration-dependent binding was observed for the other viruses, but only MVMi and MVMp-K368R bound GT3 (data not shown). The binding of each MVM virus to the three glycans tested (glycans 194, 106, and 111) is shown in Fig. 5, and their relative binding curves are shown in Fig. 6. Consistent with the glycan array data, all MVM viruses bound to glycan 3'SiaLN-LN-LN (gly-
can 194) with the highest affinity (Fig. 5). Upon visual inspection, the comparative plot for glycan 194 (Fig. 6A) showed that the affinity ranking of the viruses was in the order of MVMi = MVMp > MVMp-K368R > MVMp-I362S = MVMp-I362S/K368R. The affinities of the virulent MVMp viruses were reduced to ~60% of the MVMp levels. The dissociation rate for MVMp was slower compared with that of the virulent MVMp mutant viruses MVMp-I362S and MVMp-I362S/K368R. On the other hand, the virulent MVMp-K368R mutant had a dissociation rate close to that of MVMp (Fig. 6B). This property, combined with the slightly higher affinity to the glycan, would most likely result in a higher equilibrium affinity compared with the other MVMp mutants. The relative binding affinity to and dissociation from 3’SiaLN-LN (glycan 106) followed the same trend as observed for 3’SiaLN-LN-LN (glycan 194) for all of the MVM viruses (Fig. 6B). For GT3 (glycan 111), only MVMi and MVMp-K368R were shown to bind, with MVMi displaying approximately four times the affinity of the virulent mutant (Fig. 6C). Their dissociation rates were comparable (Fig. 6C).

DISCUSSION

The cell surface is highly abundant in sialic acid, which is utilized by numerous viruses for cellular infection. The infection of host cells by MVM is proposed to involve initial binding of the cell surface sialic acid component of a glycoprotein receptor. Using a glycan array containing ~180 different carbohydrate structures, a specific sialic acid motif recognized by MVM viruses, including allotropic strains MVMi and MVMp

FIGURE 4. Concentration-dependent glycan binding by MVMp. The concentration dependence of MVMp interaction with two of the glycans identified in the array 3’SiaLN-LN-LN (glycan 194) (A) and 3’SiaLN-LN (glycan 106) (B) were analyzed using a SPR BIAcore assay. Binding profiles are shown at virus concentrations 200, 100, 50, 25, 12.5, and 6.25 µg/ml, respectively, from top to bottom. RU, response units.

FIGURE 5. Relative affinity of glycan binding by the MVM viruses. The relative binding affinity for three representative glycans recognized in the array, 3’SiaLN-LN-LN (glycan 194), 3’SiaLN-LN-LN (glycan 106), and GT3 (glycan 111), were measured using a SPR BIAcore assay. The graphs show the results FOR MVMp (A), MVMp-I362S (B), MVMp-I362S/K368R (C), MVMp-K368R (D), and MVMi (E), respectively.
and virulent MVMp mutants isolated in SCID mice, has been identified. For the MVMp viruses, oligosaccharides with terminal sialic acid with an \( \alpha2\text{–}3 \) linkage, \( 3\text{SiaLN-LN} \), and \( 3\text{SiaLN-LN-LN} \), showed specificity (Fig. 3). The specific binding by two of the three sugars, \( 3\text{SiaLN-LN-LN} \) and \( 3\text{SiaLN-LN} \), were confirmed by a separate SPR BIAcore experiment (Figs. 4–6). The \( 3\text{SiaLN} \) motif is commonly found in \( N \)- and \( O \)-linked glycoproteins. The \( \text{sLe}^a\text{-Le}^a\text{-Le}^a \) glycan contains the \( \text{Sia-Le}^a \) motif, which is a known carbohydrate marker for cancer cells (for review, see Ref. 27). The \( \text{sLe}^a\text{-Le}^a\text{-Le}^a \) motif is reported to be involved in selectin-mediated adhesion of cancer cells to vascular endothelium and is thought to be closely associated with hematogenous metastasis of cancers (27). Interestingly, MVM is being investigated as a potential candidate for a cancer gene therapy vector, because it shows enhanced oncotropism and oncotoxicity (for review, see Ref. 28). The recognition of the \( \text{Sia-Le}^a \) motif by MVM, as identified by this glycan array analysis, provides evidence for the first time that their K368R mutant to the multisialylated GT3 glycan (Figs. 3–6) suggests that it may also be neurotropic, similar to MVMi. Amino acid 368 is one of only 14 amino acids that differ between MVMp and MVMi, and the lysine–arginine mutation reverts this residue to the MVMi type. Interestingly, comparative analysis of the three-dimensional structures of MVMi and MVMp at residue 368 showed differences in capsid surface topology and side-chain interactions between adjacent VP2 subunits that are propagated across the icosahedral 2-fold axes (10). These differences are proximal to our recently mapped sialic acid binding region on the MVMp capsid surface (11), and thus the GT3 recognition by the MVMp-K368R mutant indicates that position 368 plays a role in the extended sialic acid recognition observed for MVMi. However, because the binding affinity for GT3 is lower for the MVMp-K368R virus compared with MVMi (Fig. 6), this expanded specificity requires more than a single amino acid substitution. The virulent MVMp-I362S/K368R double mutant, which contains a change in another res-
MVMp Binding Affinity

idue that differs between MVMp and MVMi but is a valine in the latter virus, does not have the expanded specificity for GT3. Thus, the mutation to a serine at position 362 seems to have a reverting effect on the expanded specificity observed for the MVMp-K368R virus. This implies that some of the other 14 amino acids that differ between MVMi and MVMp near the sialic acid binding site (10, 11) may also be involved in the additional recognition of α2–8-linked sialic acids by MVMi.

Examples of single or few amino acid substitutions affecting receptor binding specificity and pathogenic outcomes are known for other virus families, including influenza and Theiler virus. In the hemagglutinin glycoprotein of the influenza virus, a single amino acid mutation in the receptor binding region causes the virus to switch specificity from NeuAcα2–3Gal- to NeuAcα2–6Gal-terminal residues (30). For this virus family, sialic acid usage and affinity of recognition is controlled by the receptor binding pocket of hemagglutinin (31, 32) (reviewed in Ref. 33). For Theiler virus, capsid mutations affecting sialic acid binding control neurotropism (34, 35). Future studies to identify and isolate the cell surface glycoprotein receptors for MVMp and MVMi are aimed at elucidating the role of differential glycosylation in the pathogenic phenotype of MVMi.

Glycan chain length was another determinant of MVM binding to α2–3-linked sialosides in the array. All of the MVM capsids specifically recognized only terminal sialic acid-linked α2–3 to an LN motif in an oligosaccharide that was at least five sugars long (Fig. 3). In addition, the viruses did not bind to terminal sialic acid-linked α2–6 to LN motifs. A possible explanation for the latter observation could lie with the difference in the conformation of the terminal trisaccharides resulting from this linkage. Available three-dimensional structures of sialic acid linked α2–3 or α2–6 to lactose in their protein binding pockets, such as hemagglutinin (reviewed in Ref. 33), show that the α2–3-linked trisaccharide is linear, whereas the α2–6 linkage creates a sugar chain with a kink at the α2–6 link position. Thus, the specificity for a linear motif of at least 5 residues long could arise from an interaction that requires the oligosaccharide to “lie” along a shallow groove or could indicate that a “kinked” sugar chain of comparable residues is not able to “reach” into the binding pocket on the MVM capsid surface. The latter explanation could also apply to the lack of recognition of the shorter α2–3 sialyllactosamine (3’SialLN, glycan 38) in the array. The depression at the icosahedral 2-fold axes of the MVMp capsid, where a sialic acid binding site was recently mapped, is shallow and contains charged as well as hydrophobic residues that would accommodate and stabilize a long chain of sugar molecules (11). Structural studies (by x-ray crystallography) of the MVM virus complexed with the glycans identified in the array are underway to determine the precise nature of their interactions with the amino acids in the 2-fold receptor binding pocket.

SPR BIAcore experiments independently confirmed the binding of the MVM viruses to three representative glycans identified in the glycan array (Figs. 4–6). This analysis also showed that the virulent MVMp mutants have a lower affinity for sialic acid binding compared with the wt MVMp and MVMi. These observations were in agreement with in vitro cell binding assays with A9 and NB324K in which the affinity of MVMp and the virulent MVMp mutants were compared (11). Significantly, as with the in vitro assays, the double mutant MVMp-1362S/K368R showed the lowest affinity for the glycans tested (Fig. 6). As discussed above, residues 362 and 368 are found along the wall of the 2-fold depression, close to the sialic acid binding site (Fig. 1) (11). Thus, it is likely that disrupted glycan interactions with these residues play a role in their reduced affinity compared with the MVMi and MVMp (Figs. 4–6). These observations provide further support that the virulent adaptation by the MVMp mutants is mediated through altered interactions with the host cell surface receptor.

Mutations that affect receptor sialic acid binding affinity and disease pathogenicity have been reported for a number of other viruses. For example, in polyomavirus, single amino acid changes that decrease sialic acid receptor affinity increase viral spread and mutant pathogenicity (36, 37). Interestingly, these single site mutations also cause viral polymorphisms associated with plaque phenotypes, with large plaque-forming viruses having a lower affinity for sialic acid, and are able to spread to cause tumorigenicity or virulence in mice. A similar change from the small to large plaque phenotype is observed for the virulent MVMp mutants (11). Thus, it is possible that a mechanism whereby a reduced receptor affinity results in an increase in effective spread within the host system is also responsible for the virulent phenotype of the MVMp mutants. Furthermore, it is likely that the MVMp-K368R mutant is able to spread to and infect an additional set of host cells through its expanded recognition of multisialylated glycans (Figs. 4–6). This concept of a lower receptor affinity as a determinant for virulence and pathogenicity for closely homologous members of the Parvoviridae family is novel and is under further investigation.

The glycan array and the SPR BIAcore experiments applied here utilizing whole viral capsids represent novel approaches for investigating virus-receptor interactions. The results presented will aid the design of future experiments concerning MVM host range and virulent interactions. Future structural studies of the MVM capsids and the glycans, by x-ray crystallography, are underway to investigate the structural juxtaposition that determines specific recognition and virulence.

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