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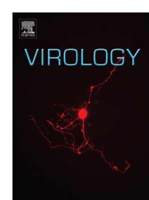

Brief Communication

Parvovirus B19 VLP recognizes globoside in supported lipid bilayers

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ABSTRACT

Studies have suggested that the glycosphingolipid globoside (Gb4Cer) is a receptor for human parvovirus B19. Virus-like particles bind to Gb4Cer on thin-layer chromatograms, but a direct interaction between the virus and lipid membrane-associated Gb4Cer has been debated. Here, we characterized the binding of parvovirus B19 VP1/VP2 virus-like particles to glycosphingolipids (i) on thin-layer chromatograms (TLCs) and (ii) incorporated into supported lipid bilayers (SLBs) acting as cell-membrane mimics. The binding specificities of parvovirus B19 determined in the two systems were in good agreement; the VLP recognized both Gb4Cer and the Forssman glycosphingolipid on TLCs and in SLBs compatible with the role of Gb4Cer as a receptor for this virus.

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Introduction

The majority of human parvovirus B19 (B19V) infections occur during childhood and are manifested as a mild self-limited rash called erythema infectiosum or the fifth disease (Servant-Delmas et al., 2010; Servey et al., 2007). However, when infecting individuals with immune or hematologic disorders, B19V can cause more severe symptoms such as acute and persistent anemia and arthropathies (Florea et al., 2007). Furthermore, infections during pregnancy may lead to hydrops fetalis, and fetal death (Yaegashi et al., 1998). The virus has a tropism for erythroid progenitor cells and the infection leads to cytotoxicity and interruption of erythrocyte production (Servant-Delmas et al., 2010).

B19V belongs to the *Erythrovirus* genus of the Parvoviridae family and is a non-enveloped single stranded DNA virus (Servant-Delmas et al., 2010). The virus capsid has a diameter of approximately 280 Å and is formed from two proteins, VP1 and VP2. The capsid has an icosahedral symmetry and consists of 60 structural subunits, of which in average 57 are VP2 and 3 are VP1. The two proteins differ through an additional N-terminal sequence of 227 amino acids on VP1.

For the past 20 years, the glycosphingolipid (GSL) globoside/globotetraosylceramide (Gb4Cer) has been considered a receptor for B19V. This stems from observations showing that the virus binds to Gb4Cer on thin-layer chromatogram (TLC) plates and that the globoside carbohydrate structure (Gb4) is essential for the cellular entry of the virus (Brown et al., 1994, 1993). Specifically, anti-Gb4 antibodies were shown to inhibit, B19V in vitro infection of bone marrow mononuclear cells (Brown et al., 1993). Moreover, purified Gb4Cer was demonstrated to block binding of the virus to erythroid cells and affect the infectivity of the virus in a hematopoietic colony assay (Brown et al., 1993). The significance of Gb4 is also supported by host genetic data: expression of Gb4 in humans is determined by the blood group P system, in which individuals of the rare blood group variant "p" lack expression of Gb4, a condition that was shown to be associated with resistance to B19V infection (Brown et al., 1994). More recent studies support a role for Gb4Cer as receptor for B19V (Bonsch et al., 2010; Leisi et al., 2013). However, these studies also demonstrated that the interaction between the virus and Gb4 induced structural changes in the viral capsid. These changes allowed the unique VP1 region to interact with a co-receptor, which was suggested to be required for firm attachment and internalization of the virus.

The role of Gb4Cer as B19V receptor has been questioned by Kaufmann et al. (2005), who reported that the virus does not bind to Gb4Cer in phospholipid membranes. This observation would imply that Gb4Cer, although binding to B19V in the chromatogram-binding assay, might not act as a bona fide receptor to B19V owing to the suggested lack of binding to lipid membrane associated Gb4Cer.

The question whether Gb4Cer constitutes a B19V receptor is not trivial. At present no functioning chemotherapy to B19V or other parvoviruses is available. The existence of a small oligosaccharide epitope as a B19V ligand opens perspectives for identifying

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the binding paratope at the viral capsid by molecular modeling, as previously described by us for noroviruses (Koppisetty et al., 2010). This may pave the way for development of tailor-made attachment inhibitors for instance based on multivalent glycan analogs (Bernardi et al., 2012), and would benefit from knowledge regarding the nature of the presentation and viral binding characteristics of Gb4Cer presented at the plasma membrane and required for virus infection.

As a complementary and versatile tool to study interactions between viruses and GSLs incorporated into supported lipid bilayers, we have previously introduced quartz crystal microbalance with dissipation monitoring (QCM-D) (Bally et al., 2012; Rydell et al., 2009). In this method, a planar silica surface acts as a solid support for the phospholipid bilayer, which is deposited on the sensor surface by spontaneous vesicle adsorption and fusion into a supported lipid bilayer. While providing unique signatures for a successful bilayer formation, the QCM-D technique is a powerful method to study the kinetics of supported lipid bilayer formation and subsequent interactions with membrane binding entities (Cho et al., 2010). The QCM-D technique is based on an oscillating piezoelectric quartz crystal and relates changes in its resonance frequency to the sensor-bound mass. Additionally, the measurements of changes in the dampening of the sensor's oscillation provide information about the viscoelastic and structural properties of the sensor-bound molecules. Thereby, adsorbed lipid vesicles, which induce high damping, are distinguishable from planar supported membranes, which induce very little damping.

We were intrigued by the opposing results regarding B19V-Gb4Cer interactions, which may originate from differences with respect to how Gb4Cer is presented to the virus. We were also well-aware of a number of studies demonstrating distinctive GSL-protein interactions depending on a number of GSL-membrane physico-chemical parameters (membrane fluidity, GSL mobility, and GSL conformation) (Bally et al., 2012; Evans and Roger MacKenzie, 1999; Lingwood, 2011, 1996; Nyholm and Pascher, 1993). Therefore, in this work, we assayed the interactions between B19V and membrane-associated Gb4Cer using QCM-D. In this way, we have characterized the interaction of a B19V VLP with GSLs incorporated into well-defined supported lipid bilayers. Binding specificity towards different GSLs was further verified using a chromatogram-binding assay and the binding pattern was in essence congruent with that of earlier studies. These data are compatible with the notion that B19V also recognizes membrane-associated Gb4Cer, reconstituting Gb4Cer as a conceivable receptor for this virus.

Results

The VLP recognizes Gb4Cer and the Forssman GSL on thin-layer chromatograms

To verify and compare the binding specificity of the B19V VP1/VP2 VLP to earlier studies, GSLs were chromatographed on thin-layer chromatograms, which were plasticized and incubated with B19V VLPs as previously described (Rydell et al., 2009). Bound VLPs were detected using antibodies and alkaline phosphatase (ALP) staining. Gb4Cer, Forssman (Gb5Cer) and neolactotetraosylceramide (nLc4Cer) GSLs were assayed for binding since they have been reported to bind B19V in similar assays in previous studies (Table 1, Fig. 1A) (Brown et al., 1993; Cooling et al., 1995). Globotriaosylceramide (Gb3Cer) was included as the negative control (Brown et al., 1993) and the VLP binding was assayed at two different concentrations (20 μg/mL and 2 μg/mL). Binding to Gb4Cer and Gb5Cer was observed for both concentrations (Fig. 1B and C). A very faint band corresponding to nLc4Cer binding was observed at 20 μg/mL, but not at 2 μg/mL. No binding to Gb3Cer was detected in any of the conditions. To exclude the possibility that any of the used antibodies cross-reacted with the GSLs, a control plate was incubated with the antibodies, but without the VLP. No binding was observed in this case (Fig. 1D).

The VLP recognizes bilayers containing Gb4Cer and Gb5Cer

Bilayer formation

To form supported lipid bilayers, POPC vesicles containing GSLs were injected into the QCM-D reaction chamber. A typical bilayer formation process is shown in Fig. 2A. At first, the vesicles adsorbed intact to the SiO_2-coated QCM-crystal which resulted in a decrease in resonance frequency, *f* (black curve), associated with the increase in mass at the sensing interface. At the same

Table 1
Names, abbreviations and structures of the glycosphingolipids investigated in this study.

Name	Abbreviation	Structure
Globotriaosylceramide	Gb3Cer	Gal α4 Gal β4 Glc
Globotetraosylceramide/Globoside	Gb4Cer	GalNAc β3 Gal α4 Gal β4 Glc
Globopentaosylceramide/Forssman	Gb5Cer	GalNAc α3 GalNAc β3 Gal α4 Gal β4 Glc
Neolactotetraosylceramide	nLc4Cer	Gal β4 GlcNAc β3 Gal β4 Glc

(yellow circle) Galactose; (blue circle) Glucose; (yellow square) *N*-acetylgalactoseamine (blue square) *N*-acetylglucoseamine.

Fig. 1. The B19V VLP recognizes globoside (Gb4Cer) and Forssman (Gb5Cer) glycosphingolipids on thin-layer chromatograms. Glycosphingolipids were chromatographed on thin-layer plates which were either stained for glycosphingolipids using anisaldehyde (A) or plasticized and incubated with VLP 20 μg/mL (B), 2 μg/mL (C) or buffer without VLP (D). Attached VLPs were detected using antibodies and alkaline phosphatase staining. The VLP recognized Gb4Cer and Gb5Cer. A weak band corresponding to nLc4Cer was observed at 20 μg/mL.

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