

# A novel approach to the study of glycolipid receptors for viruses

## Binding of Sendai virus to thin-layer chromatograms

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A method for the binding of virus to a silica gel thin-layer chromatogram is presented. After development the chromatogram is overlayed with the <sup>125</sup>I-labelled virus and the bound virus is autoradiographed. Alternatively, the unlabelled virus may be detected after exposure to monoclonal antibody and labelled anti-antibody. The Sendai virus strain used did not bind to brain gangliosides earlier proposed to be receptors, but bound to human erythrocyte gangliosides. This finding may be explained by the existence of Sendai virus variants with different receptor specificities.

<i>Sendai virus</i>	<i>Glycolipid</i>	<i>Receptor</i>	<i>Binding assay</i>	<i>Thin-layer chromatography</i>
		<i>Monoclonal antibody</i>		

### 1. INTRODUCTION

The initial stage in infections with viruses is their interaction with receptors on the host cell surface [1]. Host and tissue specificities displayed by different viruses may in part be explained by a selection at the receptor level [2]. Progress in the chemical identification of receptors has been relatively slow, mainly due to the lack of adequate techniques for the handling of solubilized amphipathic membrane components [3]. Also, traditional inhibition studies based on solubilized univalent receptors or receptor analogues may not necessarily reveal a specific virus-cell interaction based on multivalent low-affinity sites [3]. This problem has been overcome by using lipid vesicles as carriers for receptor preparations [3,4] or plastic microtiter wells to which receptors have been hydrophobically adsorbed from solution [3,5]. We have now extended the solid phase approach

employing a binding of virus particles to a thin-layer chromatogram on which one group of receptor candidates, the glycosphingolipids, has been separated. In this way a particular receptor glycolipid may be specifically detected in a mixture extracted by organic solvent from a target cell.

Sendai virus was chosen as a first object of study since brain gangliosides with the partial sequence NeuAc $\alpha$ 2 $\rightarrow$ 8NeuAc $\alpha$ 2 $\rightarrow$ 3Gal $\beta$ 1 $\rightarrow$ 3GalNAc have been shown both to bind the virus [4,5] and promote the infection [6].

### 2. MATERIALS AND METHODS

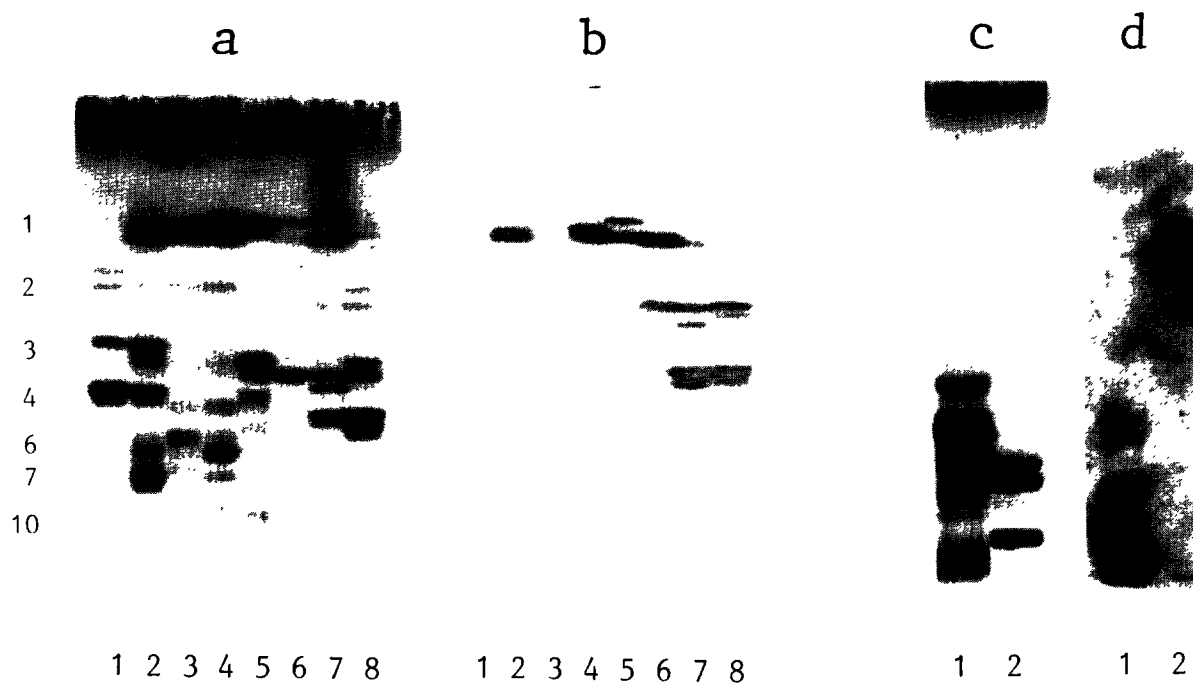
The Z strain of Sendai virus earlier used for immunological studies [7] was grown in embryonated eggs. About  $5 \times 10^8$  purified virions (about 2 mg protein) were labelled with <sup>125</sup>I in two equal portions using 250  $\mu$ l each of 50  $\mu$ Ci Bolton-Hunter reagent [8] delivered by New England Nuclear. The labelled preparation was dialyzed at room temperature against 500 ml of phosphate-buffered

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saline of pH 7.3 (PBS) for two days with two changes of buffer. The final volume of 0.5 ml was diluted with 9.5 ml PBS and had a total radioactivity of  $3 \times 10^6$  cpm. Most of the non-acid glycolipid preparations used have been described elsewhere [9–13]. Ganglioside fractions of human brain and human erythrocytes were prepared with standard methods [14].

Virus binding to glycolipids was performed on plastic-treated thin-layer plates as first used for antibody-binding [15] and later modified [16]. Glycolipids (20–40  $\mu$ g in each lane) were separated on aluminum sheets coated with silica gel 60 (Merck) using chloroform/methanol/water (60:35:8, by vol.) for non-acid (fig.1a,b) and chloroform/methanol/2.5 M ammonia (60:40:9,

by vol.) for acid glycolipids (fig.1c,d). Anisaldehyde reagent was used for chemical detection [17]. For virus binding the dried chromatogram with separated glycolipids was dipped for 1 min in 200 ml of diethylether containing 0.5% (w/v) of polyisobutylmethacrylate (Plexigum P28, Röhm, GmbH, Darmstadt) and dried for 2 min. The plate was then sprayed with PBS containing 2% bovine serum albumin (BSA) and 0.1% NaN<sub>3</sub> (solution A) and then immersed in solution A and placed in a Petri dish for 2 h. After tipping off solution A the labelled virus preparation was added (0.5 ml for each lane of about 5 mm width) to the chromatogram placed horizontally in a humidified atmosphere of a Petri dish. After incubation for 2 h the virus suspension was



**Fig.1.** Thin-layer chromatogram after detection with anisaldehyde (a) and autoradiogram (b) after binding of  $^{125}$ I-labelled Sendai virus, using for both (a) and (b) 20–40  $\mu$ g total neutral glycolipids of the following sources: human erythrocytes of blood group A (lane 1), monkey intestine of species *Macacca cynomolgus* (lane 2), human meconium of blood group A (lane 3), human meconium of blood group B (lane 4), rat small intestine (lane 5), rabbit small intestine (lane 6), guinea pig small intestine (lane 7) and dog small intestine (lane 8). The thin-layer chromatogram in (c) after detection with anisaldehyde and the autoradiogram in (d) after binding of  $^{125}$ I-labelled virus with monoclonal antibody used 30  $\mu$ g of total gangliosides of human erythrocytes (lane 1) and 15  $\mu$ g of total gangliosides of human brain (lane 2). The figures to the left of chromatogram (a) indicate the number of sugars in the neutral glycolipid bands detected. For the identity of the major ganglioside bands in fig.1c, lanes 1 and 2, the reader may consult [18] and [14], respectively. In fig.1c and d, lane 2, there is a rapid-moving glycolipid with double spot appearance which binds the virus. This is a small contamination of galactosylceramides in the brain ganglioside sample, being a positive control of binding (cf. a and b).

tipped off and the plate was washed 6 times with PBS, 1 min each time. The plate was dried and exposed at room temperature for 137 h to XAR-5 X-ray film (Eastman) using an intensifying screen.

For detection of the unlabelled virus with monoclonal antibody incubation was done for 2 h with antibody 817 [7] produced in ascites and diluted 1:100 with solution A, using about 0.5 ml for each chromatographic lane. After washing 5 times with PBS rabbit anti-mouse Fab ( $^{125}\text{I}$ -labelled  $\text{F(ab')}_2$ , about  $4 \times 10^5$  cpm/ml in solution A) was added for 2 h followed by 6 washings with PBS, drying of the plate and exposure to X-ray film [16].

### 3. RESULTS

Fig.1 documents the autoradiograms after binding of radiolabelled virus to neutral (non-acid) glycolipids (b) and unlabelled virus to ganglioside fractions (d). Unexpectedly, there was no detectable binding to the ganglioside fraction from human brain (fig.1d, lane 2). This fraction was a total ganglioside mixture (see fig.1c, lane 2 for chemical detection), which contained molecular species with the sequences  $\text{NeuAc}\alpha 2 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}$  or  $\text{NeuAc}\alpha 2 \rightarrow 8\text{NeuAc}\alpha 2 \rightarrow 3\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}$  [14] shown before to bind the virus and mediate infection [4-6]. On the other hand the virus bound strongly to slow-moving species of total gangliosides of human erythrocytes (fig.1d, lane 1) known to contain *N*-acetylneuraminic acid attached to  $\text{Gal}\beta 1 \rightarrow 4\text{GlcNAc}$  structures [18]. Also, there was an apparently avid binding to neutral glycolipids with 1, 2 and 3 sugars but not to some major glycolipids with more than 3 sugars (fig.1b, which may be compared to the same lanes after chemical detection, shown in 1a). There was a preference of binding to glycolipids with phytosphingosine or 2-hydroxy fatty acid in the lipophilic part, which migrate relatively slower than their less hydroxylated counterparts. This is evident from comparison of the 2- and 3-sugar regions, respectively, of separate lanes. The human erythrocyte sample (lane 1) did not bind and has rapid-moving species with mostly sphingosine and non-hydroxy fatty acid. On the other hand the intestinal samples showed binding and have slow-moving species due to phytosphingosine and

2-hydroxy fatty acid, as shown in lane 5 for rat [11], lane 6 for rabbit [9,12], lane 7 for guinea pig [9] and lane 8 for dog [13]. The specificity of binding concerning both the lipophilic part and carbohydrate structure has been worked out in detail using pure glycolipids (in preparation).

### 4. DISCUSSION

By treating a silica gel thin-layer plate with polyvinylpyrrolidone (PVP), protein ligands in the form of cholera toxin [19] or monoclonal antibodies [20] may be used in an overlay technique to study specific binding to glycolipids. An improved procedure has used plastic instead of PVP in order to define glycolipid epitopes in the interaction with monoclonal antibodies (see [15,16]). The role of the plastic coating may primarily be to provide a hydrophobic film from which the amphipathic glycolipids present their hydrophilic oligosaccharides with similarity to the natural cell membrane. Unspecific hydrophobic binding is prevented by final coating with BSA.

The present application to virus binding was initiated with Sendai virus because certain brain gangliosides are known to bind the virus with high affinity [4-6]. Our failure when first using the  $^{125}\text{I}$ -labelled virus to detect virus binding to these glycolipids in our assay, although using large amounts of both virus and gangliosides, may indicate that the assay conditions were not appropriate. Although unlikely, the labelling reagent may have altered the binding site of the virus hemagglutinin. We therefore used unlabelled virus and detected the virus with monoclonal antibody [7], with the same result (fig.1d, lane 2). However, there was an apparently high-avidity binding to certain gangliosides of human erythrocytes (fig.1d, lane 1) which differ in composition from brain species (see [14,18]). We are at present isolating these gangliosides for precise structure analysis.

The finding of virus binding to erythrocyte gangliosides but not to brain gangliosides may be explained by the existence of variants of Sendai virus with distinct receptor specificities. This is not unlikely, in view of the recently reported selection of influenza virus variants with specificity for  $\text{NeuAc}\alpha 2 \rightarrow 3\text{Gal}$  and  $\text{NeuAc}\alpha 2 \rightarrow 6\text{Gal}$ , respectively [21]. Also, a Sendai virus preparation was reported in [22] as unable to bind brain

gangliosides using an earlier described liposome assay which had demonstrated a strong binding [4].

The binding of the virus to neutral glycolipids with 1–3 sugars was shown both with radiolabelled ligand (fig.1b, after long exposure due to low external labelling) and after detection with antibody (not shown). After these experiments had been performed we learned about the results in [23] on the inhibition of myxovirus-induced hemolysis by some natural and synthetic simple glycolipids. A second binding site on the virus was postulated, recognizing Gal-terminating glycolipids. We may have detected the same postulated site by our direct binding assay.

The approach illustrated here is considered to be very potent for the detection of glycolipid-based receptors of viruses in general. Mixtures of potential receptors extracted from target tissues are pre-separated in the assay medium and presented on a membrane-like surface. This solid-phase multivalency of receptors should also reveal low-affinity binding sites of interest [3] in spite of shear forces produced by the washing steps. The chromatographic pre-separation of glycolipids also exposes and enables binding to very minor glycolipids that may be difficult to detect in mixed micelles. Although total lipid extracts including membrane lipids may be applied on the plate we prefer the use of purified total acid or non-acid glycolipids for a screening since one may for identification purposes directly compare the autoradiogram with chemically detected patterns (fig.1). Use of many tissue samples in one assay, different qualitatively and quantitatively, covers a very large number of receptor candidates.

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