neurons is very inefficient (11a). The existence of specific rabies virus (RV) glycoprotein (G) binding sites on the surfaces of neuroblastoma cells is demonstrated. Spodoptera frugiperda (Sf21) cells expressing G of the RV strain CVS (Gves-Sf21 cells) bind specifically to neuroblastoma cells of different species but not to any other cell type (fibroblast, myoblast, epithelial, or gloma). Attachment to mouse neuroblastoma NG108-15 cells is abolished by previous treatment of Gves-Sf21 cells with anti-G antibody. Substitutions for lysine at position 330 and for arginine at position 333 in RV G greatly reduce interaction between Gves-Sf21 cells and NG108-15 cells. These data are consistent with in vivo results: an avirulent RV mutant bearing the same double mutation is not able to infect sensory neurons or motoneurons (P. Coulon, J.-P. Ternaux, A. Flamand, and C. Tuffereau, J. Virol. 72:273–278, 1998) after intramuscular inoculation into a mouse. Furthermore, infection of NG108-15 cells by RV but not by vesicular stomatitis virus leads to a reduction of the number of binding sites at the neuronal-cell surface. Our data strongly suggest that these specific attachment sites on neuroblastoma cells represent a neuronal receptor(s) used by RV to infect certain types of neurons in vivo.

Rabies virus (RV) is a negative-strand RNA virus belonging to the rhabdovirus family, of which vesicular stomatitis virus (VSV) is the prototype. The RV glycoprotein (G) is organized in trimers protruding from the viral envelope (17, 57). Although RV multiplies transiently in muscle cells at the site of a bite and is found late in infection in secretory tissues such as the salivary glands, its growth is essentially restricted to neurons (8). Massive amounts of RV nucleocapsids accumulate in Negri bodies (38). RV inoculation of mice is generally lethal. Virulence is determined by the amino acid present at position 333 of G (15, 49, 55). For example, an R333Q mutant (i.e., whose arginine at position 333 is changed to glutamine) can infect olfactory receptor cells (27) and peripheral sensory and motor neurons (11, 14) but does not propagate to the central nervous system. Entry of motoneurons by the K330N+R333M double mutant is completely blocked, and infection of sensory neurons is very inefficient (11a).

It has been postulated that the nicotinic acetylcholine receptor (nAChR) serves as a receptor for RV (29). RV binds to the neuromuscular junction (6, 7, 28), G has sequence homology to the binding sites of some snake neurotoxins which bind to the α subunit of the muscular nAChR (30); an anti-idiotypic monoclonal antibody (MAb) raised to anti-G MAb recognizing purified nAChR binds also to several brain structures (20), and purified RV binds to the α subunit in an overlay protein binding assay (16). However, it is still unclear whether this interaction is able to mediate virus entry. In addition, RV can infect neurons which do not express nAChR (33); therefore, further molecules must act as a viral receptor(s).

In contrast to RV neuronal tropism in vivo, laboratory-passaged or fixed strains such as CVS, PV, and ERA bind in vitro to every neuronal or nonneuronal cell line tested so far, regardless of its species of origin (21, 31, 45, 48, 50, 59), making it impossible to use strategies successfully designed for cloning the receptor(s) for other viruses. These strategies rely on the existence of cell lines nonpermissive for infection and/or virus binding. In the cases of measles virus and of the transmissible gastroenteritis coronavirus, MAbs directed against cell surface proteins inhibiting virus binding have been isolated (37). Immunoaffinity purification of the viral receptor(s) was performed, and the molecule(s) was further characterized (13, 35, 60). Another strategy used for retroviruses (1, 3) and poliovirus (34), as well as for rhinovirus (19, 53) and echovirus (56), was based on transfection of a nonpermissive cell line with genes from permissive cells.

In this study, we have expressed RV G by using a recombinant baculovirus at high levels on the surfaces of insect cells. We can demonstrate specific binding of G to neuronal cell lines. This binding depends on the presence of the arginine 333 and lysine 330 and, like RV infection in vivo, is specific for neurons, suggesting that these neuronal cell lines express a specific RV receptor(s).

**MATERIALS AND METHODS**

**Cells.** Cells from Spodoptera frugiperda (Sf21) were grown in TC100 medium plus 10% fetal bovine serum (FBS) at 28°C. Mouse cells from line 3T3 (fibroblast; ATCC CCL 92) were propagated in Dulbecco's modified Eagle's medium (DMEM) ( Gibco BRL) supplemented with 10% FBS. C2C12 (mouse myoblast; ATCC CRL 1772) cells were grown in Iscove's modified Dulbecco medium (IMDM) ( Gibco BRL) supplemented with 20% FBS. They were differentiated in IMDM plus 2% heat-inactivated horse serum. Neuro 2a (derived from a clone of the C1300 tumor cell line; ATCC CCL 130) cells were grown in MEM (minimum essential medium) ( Gibco BRL) plus 10% FBS and nonessential amino acids (47). NG108-15 (a hybrid of mouse neuroblastoma N18 cells and rat glioma C6) cells were cultured in DMEM plus 10% FBS. They were used between passages 20 and 30. F11 cells (a hybrid of mouse neuroblastoma N18 cells and dorsal root ganglia) were grown in Ham's F-12 medium ( Gibco BRL) plus 15% FBS (42). Cells of the mouse neuroblastoma cell lines NIE-115 and NS20Y (2) were propagated in DMEM plus 10% FBS.
Cells of the human neuroblastoma cell lines IMR-32 (ATCC CCL 27) and SK-N-SH (ATCC HTB 11) were cultured in DMEM with 10% FBS. HeLa cells (of a human adenocarcinoma cell line) were propagated in MEM with 10% calf serum. Cells of A431, a human epithelial cell line, were grown in DMEM with 10% calf serum.

Cells of the rat glioma C6 (ATCC CCL 107) cell line were grown in F-12 medium plus 10% FBS. Cells of the PC-12 line, a rat adrenal pheochromocytoma cell line, were grown in DMEM plus 10% FBS (18).

Cells of the CV1 line, a fibroblast cell line from African green monkeys, were cultured in IMDM plus 10% FBS and β-mercaptoethanol (50 μg/ml). Cos 7 cells (CV1 cells transformed with simian virus 40 large T antigen) were grown in DMEM plus 10% FBS. Cells of the BSR line, a clone of BHK-21 cells, were grown in MEM containing HEPES (2.5 mM, D-Mann-Darby canine kidney) cells were grown in MEM plus 10% FBS plus nonessential amino acids.

**Viruses and Mabs.** The CVs and PS strains of RV as well as antigenic mutant K4-5 (11a) were propagated on BSR cells as previously described (44). The CVs strain of RV was used to infect NG108-15 cell monolayers at a multiplicity of infection (MOI) of 3 PFU/cell. These cells were used for binding assays 24 h after infection. The VSV strain Indiana was also used to infect NG108-15 cells at an MOI of 5 PFU/cell, and the cells were then used 6 h after infection. Viruses (CVS, PS, and K4-5) were concentrated according to the method of Gaudin et al. (17).

**Antigen MABS.** (30A5, 18B5, 41BC2, 40EB1, 17D2, and 8C3) recognizing different epitopes and antineuruprotein MAB 8D2 had been obtained and characterized in our laboratory (4, 26, 44).

**Recombinant baculoviruses.** Recombinant baculovirus expressing the CVS RV G was obtained from Préhuad and colleagues (43). To generate recombinant baculoviruses expressing the mutated form of RV G, Sf21 cells were infected with the following RV antigenic mutants: the R335Q in 2.5 μg of pAc80I, the R333M mutant or RLI, the K330N mutant or RK4, and the K330N + R333M mutant or K4-5 (11a). Total RNA was isolated 20 h later, and the RV G mRNA was amplified by reverse transcription-PCR with oligodeoxynucleotides 5'-GCC GGC AAC ATG CAC GCG CCT CT A and 5'-CGGG GAT TAC GCT TTA GAAA GG A CATGG TCC hybridizing to the 3' and to the complementary sequence of the 5' end of the G mRNA, respectively. The amplified DNA fragment was digested with BamHI and inserted into the transfer vector pAcYM1 (32).

Recombinant plasmids were isolated, and the sequence for each was confirmed. These recombinant plasmids were used for cotransfection of Sf21 cells with linearized lacZ baculovirus DNA from the Autographa californica nuclear polyhedrosis virus. Recombinant baculoviruses were isolated by two rounds of plaque purification with Sf21 cells. The cell line, were grown in DMEM plus 10% FBS. Cells of A431, a human epithelial cell line, were grown in DMEM with 10% calf serum. MDCK-II (Madin-Darby canine kidney) cells were grown in DMEM plus 10% FBS (18).

**Binding assays.** (i) With fluorescent Sf21 cells. Monolayers of mammalian cell lines were grown on poly-l-lysine (Sigma)-treated petri dishes (60-mm diameter) for 2 to 3 days. Noninfected Sf21 cells or Sf21 cells infected with baculoviruses at MOIs of 3 to 5 for 26 to 30 h were collected, spun, and resuspended in DMEM (107 cells/ml) of 5 μl of 5 carbohydrate-carboxyfluorescin diacetate (5-CF D) (Calbiochem) per ml were heated to 50°C, and then directly labeled the cells, which were incubated for 10 min at 28°C, diluted with DMEM, spun at 1,000 × g for 3 min, washed once with DMEM, and spun again. The cells were then respun in DMEM plus 5 mM EDTA (1 × 106 to 2 × 107 cells/ml). Two milliliters of the cell suspension were added to 1 ml of TD buffer (137 mM NaCl, 25 mM Tris-HCl [pH 7.4], 0.7 mM NaH₂PO₄, 5 mM KCl) and the radioactivity of an aliquot of the cell suspension was measured in a scintillation counter (Rackbeta 1211; LKB). After the cells were washed, the radioactivity of an aliquot of the cell suspension was measured in a scintillation counter (Rackbeta 1211; LKB). The density was brought to 1 g/cm³ by adding 1 mL of protein A-Sepharose (Sigma) at 4°C under agitation, and the beads were washed three times with TD buffer plus 1% CHAPS. Total immuno-precipitated proteins were run on a 10% polyacrylamide gel.

**RESULTS**

**NG108-15 cells bind to Sf21 cells expressing RV G (Gevs-S21 cells).** We have chosen a baculovirus expression system because G is expressed at high levels at the cell surface in its native configuration (43). The NG108-15 line is a hybrid of rat glioma C6 cells and mouse neuroblastoma N18 cells and displays motoneuron-like properties (39, 40). We monitored the binding of RV G by using rosetting assay with lepidopteran Sf21 (S. frugiperda) cells expressing RV G of the CVS strain (Gevs-S21 cells) and mouse neuroblastoma NG108-15 cells. As shown in Fig. 1A, insect cells expressing RV G and labeled by the cytoplasmic fluorescent dye 5'-CFD attached to NG108-15 cells were not bound to NG108-15 cells expressing G of the CVS strain (Gevs-S21 cells) and mouse neuroblastoma NG108-15 cells. As shown in Fig. 1A, insect cells expressing RV G and labeled by the cytoplasmic fluorescent dye 5'-CFD attached to NG108-15 cells. They did not bind to the petri dish in regions without neuronal cells. About 20% of the NG108-15 cells bound to insect cells. This may reflect the heterogeneity of the neuronal cell population. Sf21 cells not expressing G did not bind to NG108-15 cell monolayers (Fig. 1B).

**Quantitative analysis of binding between Sf21 cells and the BSR or NG108-15 cell line.** In order to quantitate binding, we infected insect cells with recombinant baculovirus at an MOI of 3 to 5. This MOI does not induce strong cytoplastic effects before 36 h; thus, the integrity of the membrane is maintained. As shown in Fig. 2, about 50% of radiolabeled Gevs-S21 cells bound to NG108-15 cell monolayers. Noninfected Sf21 cells or those infected with a recombinant baculovirus expressing β-galactosidase did not bind (<1%) to NG108-15 cell monolayers. Expression of RV G at the surfaces of insect cells does not enhance their binding to fibroblastic BSR cells, showing that the binding is specific for neuronal cells.

Figure 3 shows the curves for binding of Gevs-S21 and Sf21 cells to NG108-15 cell monolayers. The binding is linear between 0.2 × 10⁶ and 2.5 × 10⁶ Sf21 cells. Within this range, around 20% of the insect cells were able to interact with NG108-15 cells. Clumping of the Gevs-S21 cells occurred at higher concentrations (>5 × 10⁶), but even at these concentrations, we did not observe any significant binding of noninfected Sf21 cells to the neuronal monolayer.

**Internalization of the insect cells expressing RV G with anti-G Mabs inhibits binding to neuronal cells.** To ascertain whether the specificity of binding to NG108-15 cells is mediated by RV G, we examined attachment in the presence of anti-G antibodies. Because anti-G antibodies caused aggregation of Gevs-S21 cells, we were obliged to change our experi-
The binding assay was performed by the addition of dissociated, labeled NG108-15 cells to paraformaldehyde-fixed Sf21 cells. As shown in Fig. 4, under these conditions, 14% of the NG108-15 cells that were added bound to Gcvs-Sf21 cells, whereas 1.5% bound to noninfected Sf21 cells. Preincubation of the insect cell monolayers with different anti-G MAbs decreased the capacity of NG108-15 cells to bind to Gcvs-Sf21 cells by up to 90% (Fig. 4). MAb 8D2, directed against RV nucleoprotein, had no effect on the binding.

Neuronal cell lines of various origins contain specific binding sites for RV G. Gcvs-Sf21 cells were found to bind not only to NG108-15 cells but also to the mouse neuronal cell lines F11, NIE-115, and NS20Y, to Neuro 2a cells, to cells of the human neuronal cell line IMR-32, and to cells of the rat adrenal pheochromocytoma cell line PC-12 (Table 1). No specific binding was found with mouse fibroblastic 3T3 cells, differentiated or nondifferentiated myoblast C2C12 cells, human SK-N-SH cells, or epithelial HeLa and A431 cells. Interestingly, the hamster cell line BSR used to propagate RV in vitro did not mediate the binding of Gcvs-Sf21 cells. The simian CV1 and Cos 7 cells and the canine MDCK-II cell line were also negative for mediation of binding (Table 1).

These results indicate that Gcvs-Sf21 cells bind specifically to neuronal cell lines irrespective of their species of origin and of their neurotransmitter secretion. In this assay, there were differences in the binding activities (low, intermediate, or high) of the cell lines. We performed the assay on paraformaldehyde-fixed Sf21 cell monolayers because these different neuronal cell lines did not form equivalent monolayers. As shown in Fig. 5, NG108-15 cells showed the highest affinity for Gcvs-Sf21 cells (22% binding). IMR-32 and F11 cells bound quite...
efficiently (14 and 11%, respectively), whereas NS20Y and NIE-115 cells bound less efficiently. In all cases, the binding clearly exceeded that observed with noninfected cells.

Previous results have demonstrated that mutant viruses with a substitution for arginine 333 in RV G are avirulent and impaired in their capability to enter certain nerve endings, suggesting that this region is critical for binding to a cell surface receptor(s). Therefore, we generated baculovirus recombinants expressing G with various mutations: R333Q, R333M, K330N, and K330N_R333M. The expression of G at the surfaces of insect cells was analyzed 24 h after infection with the different baculovirus recombinants. Mutated G is present in equivalent amounts at the surfaces of insect cells, as determined by surface immunoprecipitation with an MAb raised to antigenic site II (30AA5) (Fig. 6) and by the ability of Gevs-SF21 cells to generate syncytia at pHs below 5.9 (data not shown). The insect cells expressing mutated RV G were then used in binding assays on NG108-15 cell monolayers. Figure 7 shows that mutations R333Q, R333M, and K330N had no effect on the binding properties of G for NG108-15 cells. The combined presence of mutations K330N and R333M in G suppressed binding to NG108-15 cells.

Purified RV can compete for Gevs-SF21 cell binding sites on the surfaces of NG108-15 cells. To exclude the possibility that binding of Gevs-SF21 cells to neuronal cell lines is not due to particular modifications of G in insect cells, we performed a competition assay with purified RV. Figure 8 shows that preincubation of NG108-15 cells with 30 μg of purified RV (CVS strain) reduced the subsequent binding of Gevs-SF21 cells to these cells by 40%; no inhibition was observed if the cells were pretreated with the double mutant (K4-5 [K330N+R333M]). Stronger inhibition (65%) was observed with 60 μg of purified RV.

### TABLE 1. Binding of SF21 or Gevs-SF21 cells to different cell monolayers

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Type</th>
<th>SF21 cells</th>
<th>Gevs-SF21 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NG108-15</td>
<td>Cholinergic neuroblastoma</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>Neuro 2a</td>
<td>Neuroblastoma</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>F11</td>
<td>Cholinergic neuroblastoma</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>NIE-115</td>
<td>Adrenergic neuroblastoma</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NS20Y</td>
<td>Cholinergic neuroblastoma</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3T3</td>
<td>Fibroblast</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C2C12</td>
<td>Myoblast</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Differentiated C2C12</td>
<td>Fibroblast</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMR-32</td>
<td>Cholinergic neuroblastoma</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>SK-N-SH</td>
<td>Adrenergic neuroblastoma</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HeLa</td>
<td>Epithelial</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A431</td>
<td>Epithelial</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>Glioma</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PC-12</td>
<td>Pheochromocytoma</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSR</td>
<td>Fibroblast</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MDCK-II</td>
<td>Epithelial</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CV1</td>
<td>Fibroblast</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cos 7</td>
<td>Fibroblast</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* –, no binding; +, low-level binding; ++, intermediate-level binding; ++++, high-level binding.

FIG. 5. Comparison of the binding activities of different neuronal cells for SF21 (–) or Gevs-SF21 (G) cells. Lepidopteran cells were fixed with 4% paraformaldehyde by use. Radiolabeled neuronal cells were collected, dissociated, and added dropwise to insect cell monolayers. Results were expressed as percentages of cell-associated radioactivity bound to the monolayers. Each value is the average of four determinations (except for the F11 cell line, for which experiments were done only in duplicate).

FIG. 6. Surface expression of mutated RV G by insect cells. SF21 cells were infected by different recombinant baculoviruses expressing mutated G. The cells were radiolabeled, and surface immunoprecipitations were performed with MAb 30AA5 as described in Materials and Methods. V, purified radiolabeled RV (PV strain); Gv, G in RV; Gb, RV G in Gevs-SF21 cells; ni, noninfected SF21 cells.
RV (PV strain). Similar inhibition was obtained if NG108-15 cells werepretreated with purified RV G rosettes (data not shown).

Following infection by the CVS strain of RV, NG108-15 cells greatly reduce binding activities for Gcvs-Sf21 cells. Infection by a virus can inhibit superinfection by the same virus. In some cases, infection may result in internalization of its receptor or may lead to saturation of the receptor(s) at the cell surface. In order to examine the effect of preinfection of neuroblastoma cells by RV upon binding activity, we infected NG108-15 cells with the CVS strain of RV for 24 h. Figure 9 shows that RV-infected NG108-15 cells were no longer able to bind to Gcvs-Sf21 cells. However, VSV-infected NG108-15 cells still showed significant binding activity. The 50% reduction in binding to VSV-infected NG108-15 cells is likely due to the severe cytotoxic effect of the virus. Following infection by RV, a decrease in the binding of Gcvs-Sf21 cells to PC-12 or Neuro 2a cells was also observed (data not shown).

DISCUSSION

Here we were able to demonstrate that RV G specifically binds to neuronal cells. We did so by development of a rosetting assay based on expression of RV G by a recombinant baculovirus on the surfaces of insect cells.

Neuronal cell lines from mice (NG108-15, F11, NIE-115, NS20Y, and Neuro 2a), humans (IMR-32), and rats (PC-12) contain specific attachment sites for RV G, whereas no receptor sites are found on epithelial (HeLa and A431), fibroblast (3T3 and BSR), glialoma (C6), or myoblast (C2C12) cells (Table 1). Treatment of Gcvs-Sf21 cells with several anti-G MAbs recognizing different epitopes abolishes binding to neuronal cell lines by steric interference. Insect cells expressing mutated G carrying the double substitution K330N+R333M bind very inefficiently to NG108-15 cells.

These results are consistent with the neurotropism of RV in vivo. Several categories of neurons of mammals having different neurotransmitters are permissive for RV. Both cholinergic (NG108-15, F11, NS20Y, and IMR-32) and adrenergic (NIE-115) neuroblastoma cells express binding sites for Gcvs-Sf21 cells, suggesting that there is no relation between neurotransmitter secretion and expression of RV-specific binding sites.

Single mutations (R333Q, R333M, or K330N) in G neither abolish the binding of Gcvs-Sf21 cells to neuronal cell lines nor impair RV infection of sensory and motoneurons after intramuscular inoculation. On the other hand, doubly mutated G (K330N+R333Q) greatly reduces the affinity of Gcvs-Sf21 cells for NG108-15 cells and also blocks infection of sensory and motoneurons (11a). The neuronal cell line NG108-15 is a derivative of a neural crest tumor and has motoneuron-like properties after differentiation (39, 40).

The RV CVS strain and its avirulent mutants can infect fibroblast cells in vitro (4a, 14, 48, 50, 54a, 59), in contrast to the pronounced neurospecificity of the G interaction observed here and in RV-infected animals. The existence of two types of receptors for RV, as postulated by others (20), may explain this apparent contradiction: a high-affinity receptor might be expressed only by neuronal cells, enabling infection of neurons in an animal (11, 14, 27) and allowing for rosette formation between Gcvs-Sf21 cells and neuroblastoma cells. We can exclude the possibility that nAChR is a high-affinity receptor capable of mediating Gcvs-Sf21 cell binding, since saturation of nAChR in PC-12 cells (20) by an MAb raised to the α subunit of the nAChR does not abrogate binding of PC-12 cells to Gcvs-Sf21 cells (data not shown). Further receptors, presumably of lower affinities, could be expressed by a variety of cells of neuronal and nonneuronal origins (50, 59). These ubiquitous low-affinity receptors could be present at high densities on the cell surface and could include the saturable binding sites on BHK cells (59). Phospholipids (51), glycolipids (10), and gangliosides (9, 52) as well as proteins (5) have been reported to serve as receptors for RV. So the nature of the high-affinity receptor on neurons remains elusive, in large part due to the difficulty in generating an in vitro assay system whose results are not made equivocal by the presence of low-affinity binding sites.

Gcvs-Sf21 cells apparently do not bind to the low-affinity receptors present on nonneuronal cells. Differences in protein modification between G’s inserted into the viral and insect cell membranes may account for this observation. G expressed by lepidopteran cells lacks sialic acid and has less complex sugar chains than G present in the viral membrane (23, 43, 54), as illustrated by the difference in their migrations in a polyacrylamide gel (Fig. 6). Alternatively, the density of G at the insect cell surface might be insufficient to mediate binding to low-affinity receptors. Binding of Gcvs-Sf21 cells to neuronal high-affinity receptors is not due to particular modifications of G in insect cells; purified RV (PV or CVS strain but not the double mutant K4-5) is able to bind to these receptors and to compete with Gcvs-Sf21 cell binding.

NG108-15 cells infected by the parental RV CVS strain do not bind to Gcvs-Sf21 cells, suggesting that after infection, the RV receptor is no longer available at the surface. This is
compatible with observations for other enveloped viruses which have developed mechanisms to prevent binding of released viruses to infected cells. In the case of influenza virus infection, the neuraminidase cleaves the sialic acid at the cell surface, thus preventing docking of the virus on the infected cell (24, 41). Measles virus and human immunodeficiency virus type 1 both down-regulate their receptors (CD46 and CD4, respectively). CD4 specifically interacts with gp120 inside the rough endoplasmic reticulum (12), inhibiting further its transport (22) and leading to its degradation (58). A different mechanism is responsible for CD46 disappearance (36) at the cell surface. Contact between cells expressing hemagglutinin and cells bearing CD46 is sufficient to trigger CD46 down-regulation in the absence of viral infection (25, 46). In the case of RV, sequestration of the receptor in infected NG108-15 cells could be responsible for the disappearance of binding activity, supporting our hypothesis that Gcs-Sf21 cell binding sites at the surfaces of neuronal cells are indeed the specific RV receptors.

We believe that there is a broad applicability of this approach to the identification of binding sites for viral G. RV is one of many viruses for which specific binding sites have remained elusive. Some viruses, such as hepadnavirus, do not replicate in culture cells; others, such as Ebola virus, are too dangerous. Some, such as herpesviruses, have several external surfaces of neuronal cells are indeed the specific RV receptors.

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