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NEUTRALIZED VESICULAR STOMATITIS VIRUS BINDS TO HOST CELLS BY A DIFFERENT "RECEPTOR"

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The addition of immune serum to vesicular stomatitis virus (VSV) results in the formation of VSV/antibody complexes which are non-infectious. Despite their being neutralized, these complexes still bind to host cells and are internalized at a rate equivalent to or greater than that observed for infectious virus. However, in contrast to infectious virus, the binding and uptake of neutralized VSV is not inhibited by phosphatidylserine and is partially sensitive to trypsin treatment. These results suggest that neutralized VSV binds to a different or additional cell binding site. By altering the VSV binding site, neutralizing antibodies may interfere with the fusogenic activity of G protein.

Immune sera can neutralize virus infectivity by several potential mechanisms: inhibition of binding, cellular uptake, or membrane penetration. In many cases (for example Newcastle disease virus, influenza virus, polio virus, vaccinia virus, and rabbit pox virus), stably neutralized viruses retain their ability to bind to the cell surface (1-6). The nature of this virus/antibody association with the cell surface, however, is not necessarily the same as that observed for infectious virus, as evidenced in some cases by alterations in uptake kinetics and elution properties (6). In order to further define the binding properties of infectious and neutralized virus, we have used VSV as a model system. VSV displays saturable binding to host cells (7). The saturable binding and uptake of VSV can be blocked by exogenous phosphatidylserine, suggesting that this phospholipid might serve as a cell membrane binding site (8). We demonstrate in this study that neutralized VSV can still bind to host cells, but that this interaction cannot be inhibited by excess phosphatidylserine. VSV/antibody complexes apparently bind to different membrane "receptors".

MATERIALS AND METHODS

Virus: VSV (New Jersey strain) was produced in Vero cells which were infected with 0.1 pfu/cell. The methodology for the labeling and purification of VSV has

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been described (8). Purified VSV labeled with S^{35}-methionine had a specific activity of 3 x 10^5 cpm/μg VSV protein and a particle/pfu ratio of 60.

Cells: Vero cells were grown to confluence in 35 mm plastic dishes in Dulbecco's modified Eagle medium containing 10% calf serum. S^{35}-VSV binding to Vero cells was performed at 37°C for 45 minutes in Dulbecco's modified Eagle medium without serum.

Binding/uptake assay: S^{35}-VSV (200ng) was added to a 1.0 ml Dulbecco's medium containing 10 μl of pre-immune rabbit serum or rabbit anti-VSV antiserum (kindly supplied by Dr. H. Arnheiter and Dr. R. Lazzarini). The mixture was incubated at 23°C for 15 minutes and then added to confluent Vero monolayers which were subsequently incubated at 37°C for 45 minutes. The cultures were then washed twice with 1.0 ml Dulbecco's phosphate buffered saline (PBS, Gibco) and incubated for 5 minutes at 37°C in PBS containing 0.25% trypsin and 0.02% EDTA. This trypsin/EDTA incubation procedure removed >90% of VSV bound to Vero cells at 4°C (7). Cells were then triturated with a Pasteur pipette, centrifuged at 3,000 g for 5 minutes at 4°C, and solubilized in 1.0 ml 1% Triton X-100. S^{35} content was quantitated by liquid scintillation.

In some experiments, phosphatidylserine was added to the S^{35} preparation just prior to incubation with Vero cells. Stock solutions of phosphatidylserine (2 mg/ml 50 mM octyl-B-D-glucopyranoside) were prepared fresh for each experiment and were diluted appropriately into Dulbecco's medium to give the stated final concentrations. The resultant phosphatidylserine liposomes were 50-100 nm in diameter and were unilamellar.

RESULTS

VSV neutralization. 200 ng S^{35}-VSV was added to 1.0 ml Dulbecco's medium containing 10 μl of either rabbit anti-VSV (N.J.) antiserum or rabbit pre-immune serum. Aliquots were then tested for residual infectivity by plaque assay on Vero cells. S^{35}-VSV treated with pre-immune serum contained 4 x 10^7 pfu/total sample while the VSV treated with anti-VSV antiserum contained <10 pfu/total sample. Antiserum caused at least a 6 log decrease in VSV plaque formation.

Association of infectious and neutralized S^{35}-VSV with Vero cells. 200 ng of S^{35}-VSV was incubated with or without 10 μl of anti-VSV antiserum exactly as described for the preceding plaque assay. The total sample was then added to confluent monolayers of Vero cells and maintained at 37°C for different time periods. The cultures were then washed twice with 1.0 ml of PBS and treated with trypsin/EDTA as described in Materials and Methods in order to remove non-internalized VSV. Fig. 1 indicates that neutralized VSV associates with Vero cells to a greater degree than infectious VSV. In three additional experiments, we observed that the apparent internalization of neutralized VSV ranged from 15-60% greater than that observed for infectious virus. Increased times of incubation of VSV with antiserum did not have a significant effect on these results.

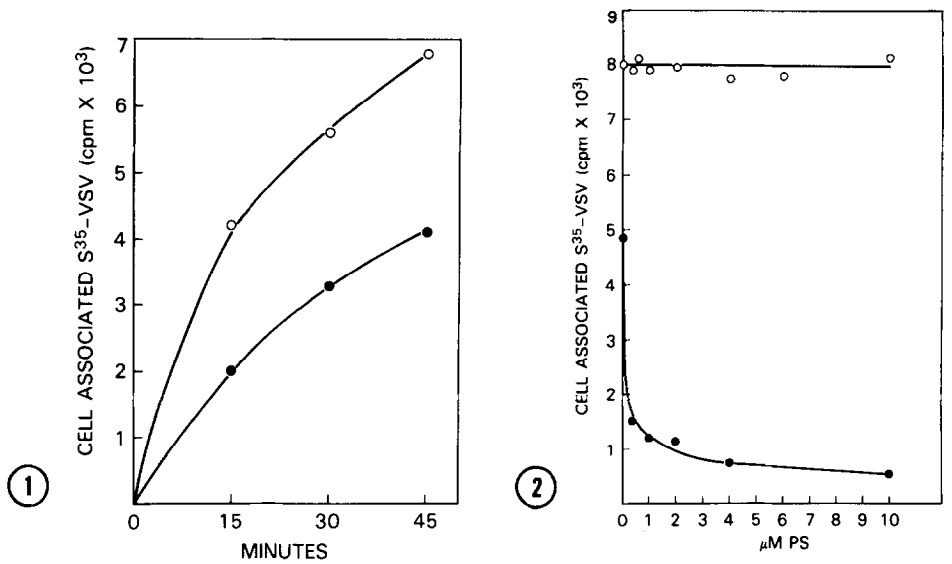

Figure 1: Trypsin/EDTA resistant association of infectious and neutralized S^{35}-VSV with Vero cells. 200 ng S^{35}-VSV was mixed with pre-immune or immune antiserum, added to confluent Vero monolayers, and assayed for cell-associated S^{35}-VSV at different times of incubation at 37°C. Neutralized VSV (o); Infectious VSV (•). Cell-association of infectious and neutralized VSV was inhibited by 90% and 70%, respectively, at 4°C.

Figure 2: Differential effect of phosphatidylserine on the uptake of infectious and neutralized VSV. The uptake of infectious (•) and neutralized (o) S^{35}-VSV was quantitated after 45 min. at 37°C as described for Figure 1. The half-maximal inhibitory concentration of phosphatidylserine for the uptake of infectious VSV was 0.1 μM (determined from other experiments). Analogous results were obtained when total cell-associated VSV was quantitated rather than the trypsin/EDTA resistant fraction.

Phosphatidylserine blocks uptake of infectious, but not neutralized, VSV. The interaction of S^{35}-VSV with host cells can be inhibited specifically by phosphatidylserine (8). 200 ng of S^{35}-VSV, with and without treatment with anti-VSV antiserum, was added to Vero cells in the presence of various concentrations of phosphatidylserine. After 45 minutes at 37°C, the cultures were washed and trypsinized as described in Materials and Methods and the amount of S^{35} measured. 10 μM phosphatidylserine inhibited the association of S^{35}-VSV with Vero cells by 90% (Fig. 2). However, neutralized VSV showed no such inhibitory effect, even at phosphatidylserine concentrations which were 100 times the half-maximal inhibitory concentration for infectious VSV. This difference in binding can best be explained by the presence of an additional or different binding site for the neutralized VSV. Consistent with this hypothesis is the finding that infectious and neutralized VSV show differences in their ability to bind to trypsinized cells. The incubation of Vero monolayers

with 50 µg/ml of trypsin for 15 min at 4°C has been shown to increase VSV binding by 27% (7). In contrast, this treatment of Vero cells caused a 25% decrease in neutralized VSV binding (control monolayers bound 4,060 cpm whereas trypsinized monolayers bound 2,990 cpm per 35 mm dish).

The identity of this new "receptor" for neutralized VSV has not been established. Attempts to compete for neutralized VSV binding with 10-50 fold excess amounts of immunoglobulin Fc fragments, goat immunoglobulin, and rabbit immunoglobulin have not been successful. It is possible that the VSV G protein/antibody interaction exposes new domains of G protein (or antibody) which can mediate cell binding.

DISCUSSION

Neutralizing antibodies evidently do not prevent the uptake of VSV. On the contrary, there is a variable stimulation of VSV uptake which is effected by such antibodies, similar to that observed for poliovirus (3). Whether this stimulation results from the particular properties of a new receptor for VSV, from some degree of VSV aggregation, or from the properties of a different cellular uptake mechanism is unknown. The finding that phosphatidylserine inhibits infectious VSV uptake but not neutralized VSV uptake suggests that different cell receptors may be involved. However, it will be important to define the precise morphological route of entry for neutralized VSV. As shown previously (9-13), infectious VSV is opportunistic and utilizes the "receptor-mediated endocytic" pathway for gaining access to the cell interior. VSV has been shown to bind to phosphatidylserine and possibly interacts with this lipid at the cell surface (8). For convenience, we have referred to phosphatidylserine as a possible "receptor" for VSV, yet realize that this term may not yet be particularly appropriate for virus/cell interactions and especially for protein/lipid interactions. By electron microscopy, VSV appears to bind or to quickly associate with specialized regions of the plasma membrane termed "coated pits". Following this localization, VSV is then transferred to an intracellular, uncoated vesicle population (endosomes). These endosomes are quickly acidified (14) and it is speculated that a resultant, conformational change in VSV G protein facilitates virus: cell membrane fusion. Interestingly, acidic pH has been shown to effect G protein-mediated cell fusion (15). Presumably,

neutralized viruses would remain within the acidic endocytic vesicles and eventually be transferred to lysosomes and degraded. A similar finding has been reported for neutralized vaccinia virus (5).

The inability of neutralizing antiserum to block VSV uptake is paralleled by the inability of such antiserum to block the association of purified G-protein with the cell surface (16). It is not known, however, whether G-protein aggregates display the same changes in receptor binding consequent to interaction with anti-G protein antibodies.

The observation that antiserum can also neutralize pre-bound VSV (12) suggests that VSV can bind to some degree to phospholipids and yet still be rendered non-infectious by antibodies. This could result from the ability of the antibodies to prevent or mask conformational changes in VSV G protein which facilitate the additional lipid interactions necessary for membrane fusion.

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