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BINDING RATE OF RABIES VIRUS TO ALPHA INTERFERON PRIMED MOUSE NEUROBLASTOMA CELLS

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Summary. - Mouse neuroblastoma cells (MNA) were primed with 20 I.U. of alpha interferon (Mu IFN-α) prior to exposure to the Challenge Virus Standard strain of rabies virus (CVS). Saturation of CVS receptor sites occurred when 0.71 μg of ^{3}H-CVS protein bound to 20,000 MNA cells. After 180 min incubation time, the amount of viral protein attributed to specific binding was estimated to be 0.45 μg. Mu IFN-α treatment of MNA cells did not increase the number of specific cell receptor sites to CVS but it did significantly increase the rate that CVS was able to bind to cell receptors. The amount of time required to reach saturation of MNA cell receptors to CVS decreased from 120 min in untreated cells to 30 min in Mu IFN-α primed MNA cells. Although treatment with Mu IFN-α increased the binding rate of rabies virus to MNA cells, the virus was unable to complete a productive infection 48 hrs after the Mu IFN-α was removed.

Key words: rabies; CVS; interferon; receptor sites

Introduction

Rabies virus is highly sensitive to the antiviral effects of interferon (Stewart and Sulkin, 1966; Hilfenhaus *et al.,* 1975, 1977; Honda *et al.,* 1985). During the progression of a natural or induced rabies virus infection there is a concurrent increase in the production of interferon, notably in the brain (Wiktor *et al.,* 1976; Marcovistz *et al.,* 1984). Both *in vivo* and *in vitro* experiments indicate that interferon must come in physical contact with the cell surface in order to illicit an antiviral response. When the cell surface is bypassed using *in vitro* methods, interferon fails to illicit an antiviral response (Faltynek and Baglioni, 1984; Blalock and Smith, 1986). The attachment of both interferon and rabies virus is believed to be lipid associated. In fact both substances have been reported to bind to membrane gangliosides (Besancon and Ankel, 1974; Vengris *et al.,* 1976; Conti *et al.,* 1988; Faltynek *et al.,* 1988). Because they are both associated

with membrane gangliosides, it is possible that interferon and rabies virus may share a common binding site or a partial binding site to a lipid component located on the cell membrane. It is also possible that one substance may alter the binding behavior of the other. This report investigates the effect that interferon has on the rabies virus receptor sites located on mouse neuroblastoma cells.

Materials and Methods

Cells and virus. Mouse neuroblastoma cells (MNA) from the cell line of A/J ($H\text{-}2_2$) (Wiktor *et al.*, 1977) were obtained from the Centers for Disease Control in Atlanta Georgia. MNA cells were grown in Eagle's minimum essential media supplemented with 10 % fetal calf serum, 2% L-glutamine, and 2% minimum essential media vitamins (EMEM). The Challenge Virus Standard (CVS) strain of rabies virus was used throughout this study. Virus was produced in MNA cells and purified as previously described (Sokol, 1969). Labelled CVS was prepared by infecting monolayers of MNA cells with CVS for one at a multiplicity of infection (MOI) of 0.1. Infected cell layers were maintained in Eagle's minimum essential media deficient in L-Leucine supplemented with 0.1 % bovine serum albumin (BSA) and 10 370 MBg/ml of ^{3}H L-Leucine. Viral protein was determined using a Pierce micro MCA protein assay (Rockford, Illinois). Viral titre was calculated in fluorescing focus units (FFU) as described (Smith *et al.*, 1973, Wunner, 1985). Purified unlabelled and labelled CVS were stored at -80 °C until needed.

Interferon. Purified mouse alpha interferon (Mu IFN-α) with a molecular weight of 27-28 kD was purchased from Lee Biomolecular Research Laboratories, Inc. (San Diego, California). MNA cells were assayed to confirm their susceptibility to the antiviral effects of Mu IFN-α as previously reported (Marcovistz *et al.*, 1987). MNA cells were treated with 20 International Units (I.U.) of Mu IFN-α, incubated for 20 hrs at 37 °C and then infected with unlabelled CVS at a MOI of 10 FFU/cell. At 24 and 48 hrs cells were fixed, stained with FITC-labelled anti-rabies antibody, and examined for fluorescence.

Saturation of MNA cell receptor sites. MNA cells were seeded in 96 well flat bottom plates at a concentration of 20,000 cells/well and incubated overnight at 37 °C. Plates containing monolayered cells were removed from the incubator and cooled to 4 °C. All further experimental procedures and reagents were maintained at 4 °C. The cell layer was washed twice with 0.01 mol/l physiological saline buffer pH 7.5 (PBS). After removal of the PBS, 1.8 μg, 4.5 μg, 14.4 μg, 21.6 μg, and 28.7 μg of purified ^{3}H-CVS were added to individual wells and incubated at 4 °C for 90 min. The ^{3}H-CVS was removed and the cell layer washed gently with PBS. The PBS was removed and the MNA cells with attached ^{3}H-CVS were suspended in 0.05 ml of 0.1 N NaOH. The suspended MNA cells with cell-associated ^{3}H-CVS were placed in vials containing four ml of Beckman Ready Safe liquid scintillation fluid and counted for one minute in a Beckman LS1701 Liquid Scintillation counter. Viral titer and the number of counts per minute (CPM)/μg of purified ^{3}H-CVS were calculated. The amount of cell-associated viral protein was estimated by dividing the total CPM attached to the cells by the CPM/μg of purified ^{3}H-CVS. The experiment was conducted in triplicate and mean values were calculated for each volume of ^{3}H-CVS added to the cells.

Determination of specific and non-specific binding. Non-specific binding of ^{3}H-CVS was determined using a cold competition assay. MNA cells were seeded in 96 well microtiter plates at a concentration of 20,000 cells/well. Cells were allowed to monolayer overnight at 37 °C and then placed on ice at 4 °C for the remained of the experiment. The EMEM was removed and the cell layer was washed with cold PBS. Cells were infected in triplicate with either 1,000 μg of unlabelled and 12.5 μg of ^{3}H-CVS or with 12.5 μg of ^{3}H-CVS alone. At 30, 60, 120, and 180 min the virus inoculum was removed and the cell layer washed with cold PBS. Cell associated virus was collected as described above and counted for one min in a scintillation counter. The amount of

^{3}H-CVS that bound to the cells in the presence of excess unlabelled CVS was considered to be non-specific binding. Specific binding of ^{3}H-CVS was calculated by subtracting the amount of virus bound non-specifically from the total amount of cell-associated ^{3}H-CVS.

Binding of CVS to Mu IFN-α treated MNA cells. Twenty I.U. of Mu IFN-α was incubated with 20,000 MNA cells at 37 °C for 20 hrs. After incubation the monolayer was placed on ice and the monolayer was washed with cold PBS. Specific and non-specific binding of ^{3}H-CVS were determined as described above. Samples were taken at 1, 5, 15, 30, 60, 120, and 180 min and counted for one min in a scintillation counter.

Binding of CVS to MNA cells in the presence of Mu IFN-α. MNA cells were seeded in two 96 well flat bottom plates and incubated overnight at 37 °C. After incubation the monolayer was placed on ice, the EMEM was removed and the monolayer was washed with cold PBS. The PBS was removed and replaced with 580 I.U. of Mu IFN-α and 12.5 μg of ^{3}H-CVS or 12.5 μg of ^{3}H-CVS alone. Samples from the untreated cells were taken at 5, 15, 30, 60, 120, and 180 min. Samples were processed as described above and counted in a scintillation counter for one min.

Assay for productive infection. MNA cells were seeded in eight well chamber Lab-Tek slides (Nunc Incorporated, Naperville Illinois) in the presence of 20 I.U. of Mu IFN-α at a concentration of 20,000 cells/well. Cells were incubated overnight at 37 °C and then placed on ice, the EMEM was removed and the monolayer was washed with PBS. Control slides were also prepared as described above except that no Mu IFN-α was used. Each well was exposed to 12.5 μg of CVS for 60 min. The excess CVS was removed and the cell monolayer was washed extensively with cold PBS. After the last wash was removed, the cell layer was covered with viral growth media consisting of Eagle's minimum essential media supplemented with 0.1% BSA, 10% L-glutamine, and 2% minimum essential media vitamins. Viral growth media was removed from individual wells and assayed for rabies virus production at 0 hrs and every four hours until the experiment was terminated at 48 hrs.

Results

Specific and non-specific binding of ^{3}H-CVS to MNA cells

Saturation of receptor sites to rabies virus occurred when 0.71 μg of ^{3}H-CVS protein bound to MNA cells after 120 min Sixtythree percent or 0.45 μg of this viral protein was attributed to specific binding. Saturation of receptors to ^{3}H-CVS was reached at 120 min and the amount of viral protein bound to MNA cells did not increase before the experiment was terminated at 180 min (Fig. 1).

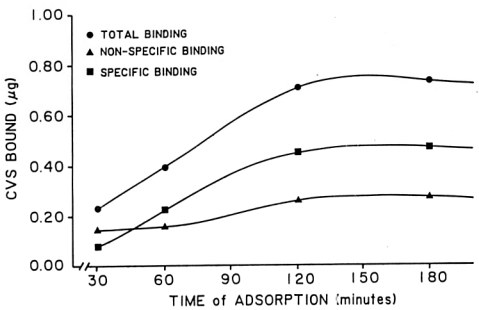

Fig. 1
Saturation of rabies virus receptor sites on mouse neuroblastoma cells by ^{3}H-CVS
Mouse neuroblastoma cells were incubated at 4 °C with increasing amounts of ^{3}H-CVS for 90 min. The amount of ^{3}H-CVS bound to the cells in the presence of excess (1000 μg) unlabelled CVS was considered to be nonspecific binding. Cells were washed with cold physiological saline and counted for cell-associated radioactivity. Each data point represents the mean of three experiments.

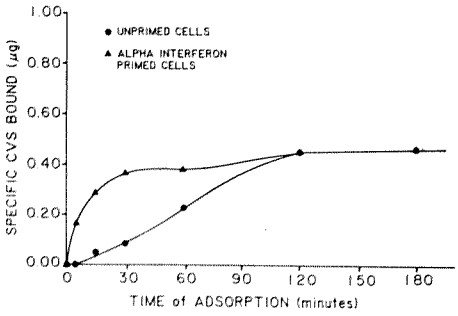

Fig. 2
Specific saturable binding of ^{3}H-CVS to mouse neuroblastoma cells
Cells were incubated at 4 °C with 12.5 μg of ^{3}H-CVS or 1000 μg of unlabelled CVS and 12.5 μg of ^{3}H-CVS. Cells were washed with cold physiological saline and counted for cell-associated radioactivity at 30, 60, 120, and 180 min postexposure. Each data point represents the mean of three experiments

Influence of Mu IFN-α on the binding of ^{3}H-CVS to MNA cells

Treatment of MNA cells with Mu IFN-α for 20 hrs prior to exposure to ^{3}H-CVS caused an initial increase in viral binding (Fig. 2). The total amount of ^{3}H-CVS bound to Mu IFN-α primed cells reached saturation at 30 min post--exposure. The mean amount of total ^{3}H-CVS needed to saturate primed cells after 30 min was 0.68 μg (Table 1).

Association of increased binding to productive infection

Unprimed MNA cells began to produce rabies virus 32 hrs after they were exposed to CVS. The virus titer continued to rise until the experiment was terminated at 48 hrs. A productive infection did not occur during the 48 hr sample time in MNA cells pretreated with Mu IFN-α and then exposed to CVS.

Table 1. Saturation of MNA cells primed with Mu IFN-α

Time of adsorbance (minutes)	Micrograms of ^{3}H-CVS protein		
	Total	Non-specific	Specific
1	0.19	0.03	0.16
5	0.20	0.03	0.17
15	0.36	0.07	0.29
30	0.68	0.31	0.37
60	0.68	0.29	0.38
120	0.69	0.29	0.40
180	0.72	0.30	0.42

^{3}H-CVS protein bound to MNA cells primed with 20 I.U. of Mu IFN-α for 20 hrs. Cells were incubated with ^{3}H-CVS at 4 °C. At each sample time cells were washed with cold PBS and assayed for cell-bound radioactivity. Each data value represents the mean of three experiment.

Discussion

Mouse neuroblastoma cells provide a useful model to study the interaction that occurs between Mu IFN-α and rabies virus during viral attachment. MNA cells have surface receptors to both Mu IFN-α and rabies virus. When these cells are primed with Mu IFN-α an antiviral response is invoked that prevents rabies virus from developing into a productive infection. Two criteria necessary to validate the existence of cell receptors to a particular protein, are saturability and competition for cell receptor sites. Saturability of rabies virus receptor sites on the mouse neuroblastoma cell line NS 1300 has been described earlier (Wunner *et al.*, 1984). This report used both saturability and competition of rabies virus receptor sites to investigate the influence of Mu IFN-α on MNA cells.

When MNA cells were primed with 20 I.U. of Mu IFN-α for 20 hrs prior to exposure to rabies virus a significant difference in the initial rate of viral attachment to the cell membrane was evident. This increase in binding rate was not due to the physical presence of interferon because the increased binding rate did not occur immediately when MNA cells were exposed to Mu IFN-α in the presence of CVS. The MNA cell membrane had to be exposed to Mu IFN-α several hours before the binding rate increased. Therefore Mu IFN-α must have induced a change in the cell membrane in order to cause the binding rate to increase. It is possible that interferon treatment altered the charge on the membrane surface as has been reported previously (Premecz *et al.*, 1989). However, interferon is known to produce more complex membrane changes than electrostatic fluctuations in various cell lines. The mechanisms by which these membrane alterations influence viral attachment and antiviral activity is unknown. Human amniotic cells exposed to human interferon-α produce a number of cellular enzymes including phospholipase C and phospholipase A_2 that induce antiviral activity by altering the cell membrane (Premecz *et al.*, 1989). A similar circumstance may occur in MNA cells exposed to Mu IFN-α. The membrane changes that occurred when MNA cells were primed with Mu IFN-α did not increase the amount of rabies virus that bound to specific receptor sites on the cell surface at the end of the experimental time period. This suggests that the number of cell receptor sites to rabies virus is not increased by Mu IFN-α.

The data obtained in this report indicate that Mu IFN-α increases the binding rate of rabies virus to MNA cells by inducing membrane changes. The antiviral influence on cell metabolic products induced by additional cellular enzymes produced by exposure to interferon have been documented but the effect these enzymes have on the cell membrane is just beginning to be investigated. Because rabies virus has an affinity for neuronal cells *in vivo* any alterations that are induced by interferon in these cell types may have implications in the use of interferon for treatment of persons exposed to rabies. Further investigation into possible alterations that occur to membrane lipids when MNA cells are

primed with Mu IFN-α will help to identify specific membrane receptors to rabies virus and may lead to more useful treatment for persons exposed to rabies.

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