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Fusion Properties of Cells Infected with Human Parainfluenza Virus Type 3: Receptor Requirements for Viral Spread and Virus-Mediated Membrane Fusion

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Cells can be persistently infected with human parainfluenza virus type 3 (HPF3) by using a high multiplicity of infection (MOI) (≥5 PFU per cell). The persistently infected cells exhibit no cytopathic effects and do not fuse with each other, yet they readily fuse with uninfected cells. We have previously shown that the failure of the persistently infected cells to fuse with each other is due to the lack of a receptor on these cells for the viral hemagglutinin-neuraminidase glycoprotein, and we have established that both fusion and hemagglutinin-neuraminidase proteins are needed for cell fusion mediated by HPF3. We then postulated that the generation of persistent infection and the failure of cells infected with HPF3 at high MOI to form syncytia are both due to the action of viral neuraminidase in the high-MOI inoculum. In this report, we describe experiments to test this hypothesis and further investigate the receptor requirements for HPF3 infection and cell fusion. A normally cytopathic low-MOI HPF3 infection can be converted into a noncytopathic infection by the addition of exogenous neuraminidase, either in the form of a purified enzyme or as UV-inactivated HPF3 virions. Evidence is presented that the receptor requirements for an HPF3 virus particle to infect a cell are different from those for fusion between cells. By treating infected cells in culture with various doses of neuraminidase, we demonstrate that virus spreads from cell to cell in the complete absence of cell-cell fusion. We compare the outcome of HPF3 infection in the presence of excess neuraminidase with that of another paramyxovirus (simian virus 5) and provide evidence that these two viruses differ in their receptor requirements for mediating fusion.

The envelope of human parainfluenza virus type 3 (HPF3) contains two viral glycoproteins, the hemagglutinin-neuraminidase (HN) and fusion (F) proteins. Infection of cells by HPF3 is initiated by attachment of the virus to the host cell through interaction of the HN glycoprotein with a neuraminic acid-containing molecule on the host cell surface. Penetration and uncoating of the virus occur by fusion of the viral envelope with the plasma membrane of the cell, resulting in the release of the viral nucleocapsid into the cytoplasm. The precise roles of each of the viral glycoproteins, HN and F, in mediating this critical fusion event are not known. Although there is ample evidence that the F protein is critical for the fusion process (1), we have reported that the HN glycoprotein is also essential for membrane fusion mediated by HPF3 (13). Using a tissue culture model of persistent infection with HPF3, we determined that interaction of HN with its sialic acid receptor is required in order for F to promote membrane fusion.

Persistent infection with HPF3 was readily established in CV-1 cells by infecting the cells at a multiplicity of infection (MOI) sufficient to infect all of the cells in the culture (≥5 PFU per cell). Under these conditions, the cells do not exhibit the cell-cell fusion and syncytium formation that is the hallmark cytopathic effect (CPE) of this virus and upon passage yield persistently infected (PI) cell cultures (12, 24). The cells remain free of CPE even after passage for several years, while shedding infectious virus and defective interfering particles into the medium (11). Although the PI cells contain both HN and F in their plasma membranes, they are entirely refractory to self-fusion yet are able to undergo rapid and complete fusion when seeded with uninfected cells. We showed that the critical molecule supplied by the uninfected cells is surface sialic acid and that interaction of HN with its sialic acid receptor is critical for fusion of the PI cells; this implied that the failure of the PI cells to fuse with each other is due to a lack of sialic acid-containing receptors for the viral HN glycoprotein (13). These sialic acid residues are thought to be removed from the surfaces of PI cells by the action of the viral neuraminidase present in the plasma membrane of PI cells.

The finding that interaction of HN with its receptor on an adjacent cell is required for virus-induced cell fusion suggested a mechanism for the rapid establishment of persistence following high-multiplicity infection. We hypothesized that the high levels of viral neuraminidase present in the high-MOI inoculum might remove the cell surface sialic acid receptors for the HN glycoprotein, preventing virus-induced cell fusion and preventing the development of CPE. This could then allow the development of persistent infection. In this report, we provide evidence to support this mechanism for the establishment of persistent infection with HPF3 in tissue culture. We present information about the sialic acid receptor requirements for the spread of HPF3 and for HPF3-mediated membrane fusion and provide evidence that the sialic acid receptor requirements for an HPF3 virus particle to infect a cell by fusion of the viral envelope with the plasma membrane are different from those for fusion of an infected cell with an uninfected cell.

MATERIALS AND METHODS

Viruses and cells. CV-1 cells and BHK cells were grown in monolayers in Eagle's minimal essential medium supplemented with 10% fetal bovine serum. Stocks of HPF3 were

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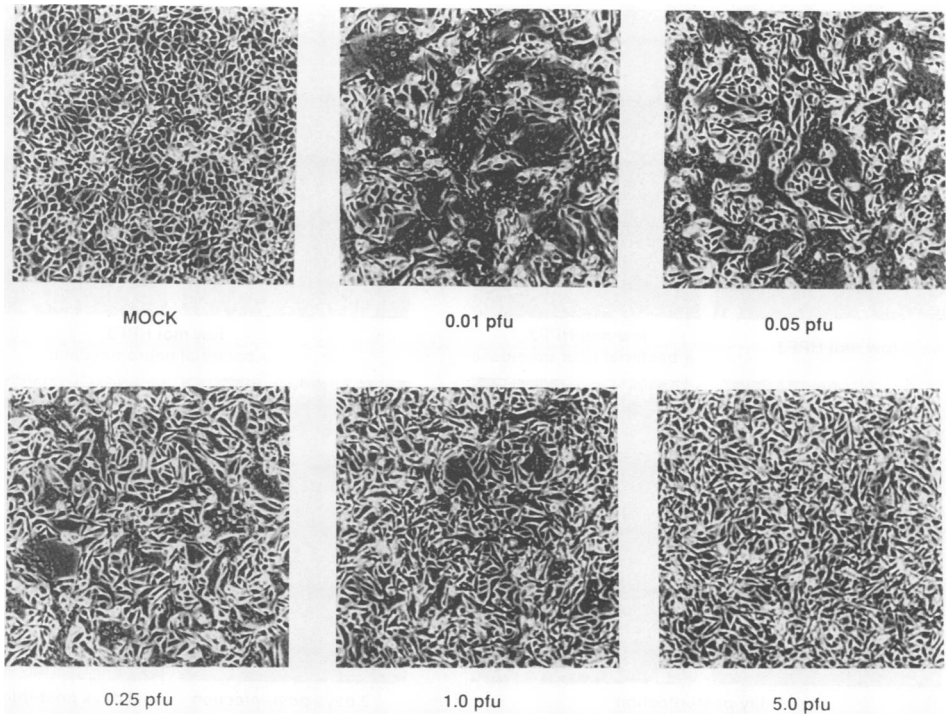

FIG. 1. Effect of MOI of HPF3 on the degree of CPE in a monolayer of CV-1 cells. Monolayer cultures of CV-1 cells were infected with various MOIs of plaque-purified HPF3, incubated at 37°C, and photographed at 18 h after infection. The MOI used in each experiment is indicated below each photograph.

made in CV-1 cells from virus that was plaque purified four times. Virus titer was determined by plaque assay with CV-1 cells. The cells were infected by aspirating the medium, washing the monolayers with medium lacking serum, and then adding medium lacking serum with various amounts of HPF3 to the cells as described in the text. After incubation at 37°C for 60 min, the medium was aspirated and medium lacking serum was added back. Simian virus 5 (SV5) was a gift from Robert A. Lamb. The cells were infected as described for HPF3.

Neuraminidase treatment. Monolayer cultures of infected cells were treated with neuraminidase (from *Clostridium perfringens*; Sigma Chemical Co., St. Louis, Mo.) by washing the cells with medium lacking serum and then adding serum-free medium containing neuraminidase to the cells. Uninfected cells were pretreated with neuraminidase for 90 min and then washed and infected with virus. Medium containing neuraminidase was then added to these cells.

UV irradiation of virus. Virus was treated to eliminate its infectivity by irradiation with 254-nm-wavelength light at a distance of 10 cm for 7 min on ice. The loss of infectivity was confirmed by plaque assay. Cells were infected with HPF3 at an MOI of 0.1 PFU per cell, an amount of UV-irradiated virus equivalent to an MOI of 10 was then added to the cells, and incubation was continued for 90 min more. Medium lacking serum was then added, and the cells were photographed the next day.

Hemadsorption assays. Monolayers were washed with cold medium lacking serum and then incubated with human erythrocytes at 4°C for 75 min. Nonadherent cells were removed by washing them with cold medium, and the cells were photographed through a phase-contrast microscope.

Assays of released sialic acid. Monolayer cultures were washed twice with medium lacking serum and phenol red and then incubated with the same medium containing various amounts of *C. perfringens* neuraminidase as indicated in the text. After 90 min, the medium was removed and assayed for the presence of sialic acid by high-performance liquid chromatography (HPLC) as described elsewhere (17). To assay the amount of sialic acid released by UV-irradiated virus treatment, it was necessary to pellet and wash the virus after UV irradiation to remove the sialic acid that was present in the virus preparations. The pelleted virus was resuspended in medium lacking serum and phenol red.

RESULTS

Effect of MOI on CPE caused by HPF3. The degree of cytopathology that occurs in a monolayer of cells infected with HPF3 depends on the MOI. Figure 1 illustrates the cytopathic effects induced after infection with several different MOIs of HPF3. The first photograph in Fig. 1 is of a monolayer of uninfected CV-1 cells. The second photograph shows a typical infection with the virus at a low MOI, with large areas of fusion and syncytia present. Each of the next photographs in Fig. 1 shows the CPE at the same time after infection, with progressively higher inocula. As the MOI increased, the CPE decreased. At the highest MOI used, 5 PFU per cell, there was no CPE. These cells, however, were persistently infected with HPF3, as demonstrated by the expression of viral genes in these cells and the release of infectious virus from these cells (11, 12).

Effect of low-level exogenous neuraminidase on CPE produced by HPF3: spread of infection without cell fusion. We hypothesized that the neuraminidase present in a high-MOI HPF3 inoculum results in removal of the cell surface sialic acid, preventing cell-to-cell fusion and thus preventing the development of virus-induced CPE. This would explain why

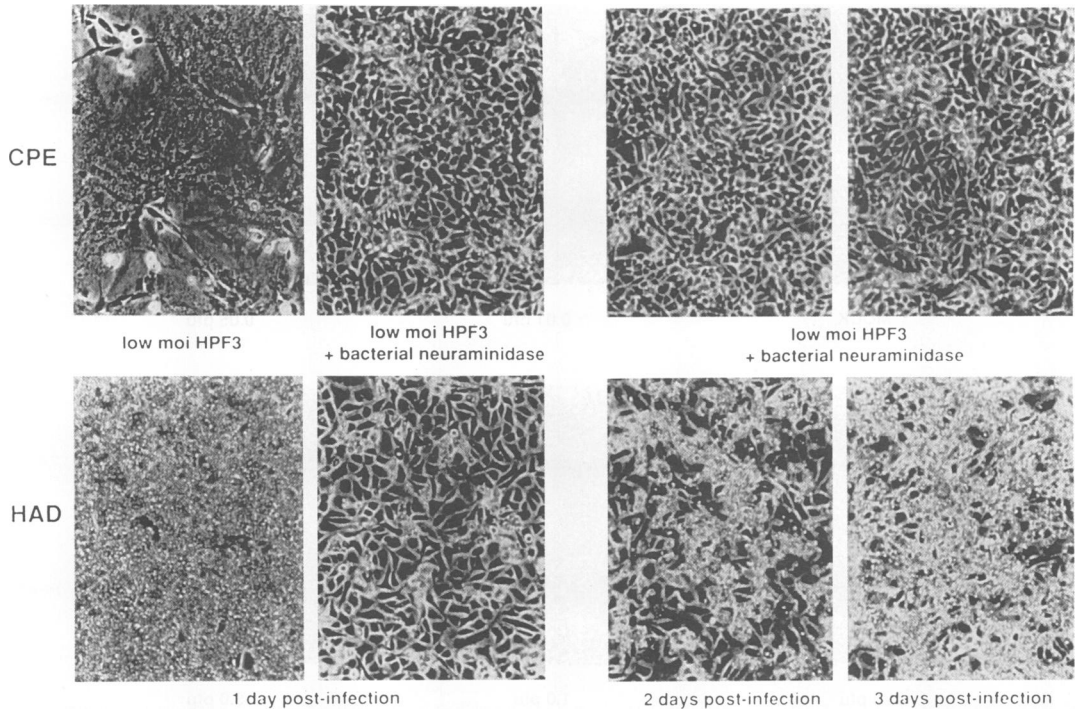

FIG. 2. Effect of low-level exogenous bacterial neuraminidase on CPE produced by HPF3: spread of infection without cell fusion. Monolayer cultures of CV-1 cells were infected with HPF3 at a low MOI (0.01 PFU per cell). The first column of photographs shows the CPE and degree of hemadsorption (HAD) 1 day after infection, after addition of HPF3 alone at a low MOI. The second column of photographs shows the CPE and HAD at the same time after infection (1 day); however, in this experiment 0.1 U of *C. perfringens* neuraminidase was added after viral infection. The final two columns of photographs show the CPE and HAD of the neuraminidase-treated infected cells at 2 and 3 days after infection. Progression of the infection can be monitored by observing the increase in HAD-positive cells.

the CPE decreases as the MOI increases. To test this hypothesis, CV-1 cells were infected with HPF3 at a low MOI and then treated with bacterial neuraminidase. Figure 2 shows CV-1 cell monolayers after infection with a low MOI of HPF3. The first column of photographs demonstrates extensive cell fusion 24 h after infection, and all of the cells in the monolayer were hemadsorption positive. In contrast, in the second column of photographs in Fig. 2, treatment of the infected cells with neuraminidase prevented cell fusion and only a small percentage of the cells were hemadsorption positive. When these neuraminidase-treated infected cells were incubated longer, the infection spread throughout the culture, as indicated by the increase in the percentage of cells exhibiting hemadsorption at days 2 and 3 after infection; yet no fusion was observed. Exogenous neuraminidase prevented cell fusion, but virus was able to spread rapidly throughout the culture even in the total absence of cell fusion.

To model more closely the conditions present during high-MOI infection with HPF3, virus was UV irradiated to eliminate its infectivity and was then used as the source of neuraminidase. The results of this experiment are shown in Fig. 3. Cells were infected with a low MOI of infectious virus, and then an amount of UV-irradiated virus equivalent to that in a high-MOI inoculum was added to the cells. The addition of the UV-irradiated virus to the cells completely prevented the infectious virus in the low-MOI inoculum from inducing cell fusion; there were no syncytia in the cell monolayer. These two experiments reveal that the important feature of high MOI for preventing CPE is, in fact, the presence of high levels of neuraminidase.

In both cases of infection at low multiplicity in which fusion was prevented by adding exogenous neuraminidase (either bacterial or viral), virus spread rapidly through the culture in the absence of cell fusion and persistent infection was established, resulting in PI cell cultures. These cultures remain PI, as determined by the expression of viral genes in these cells and the release of infectious virus, and are indistinguishable from PI cultures produced by a high-MOI inoculum of HPF3 (12).

Quantitation of sialic acid released by neuraminidase treatments. In order to show that the neuraminidase treatments resulted in removal of sialic acid from the cell surfaces, we assayed the amount of sialic acid released into the medium

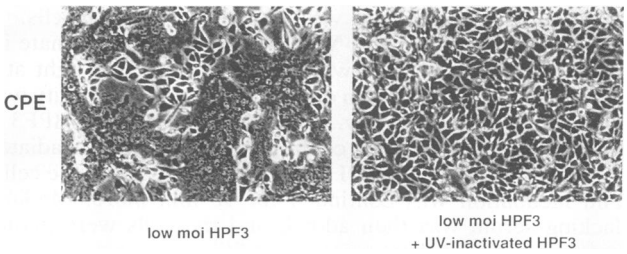

FIG. 3. Effect of UV-inactivated HPF3 on CPE produced by low-MOI infection with HPF3: use of noninfective virus as a source of viral neuraminidase. Monolayer cultures of CV-1 cells were infected with HPF3 at a low MOI (0.01 PFU per cell) and photographed 1 day after infection. In the experiment shown in the photograph on the right, an amount of UV-irradiated virus equivalent to that in an infection with an MOI of 10 was added to the cells after the low-MOI infection.

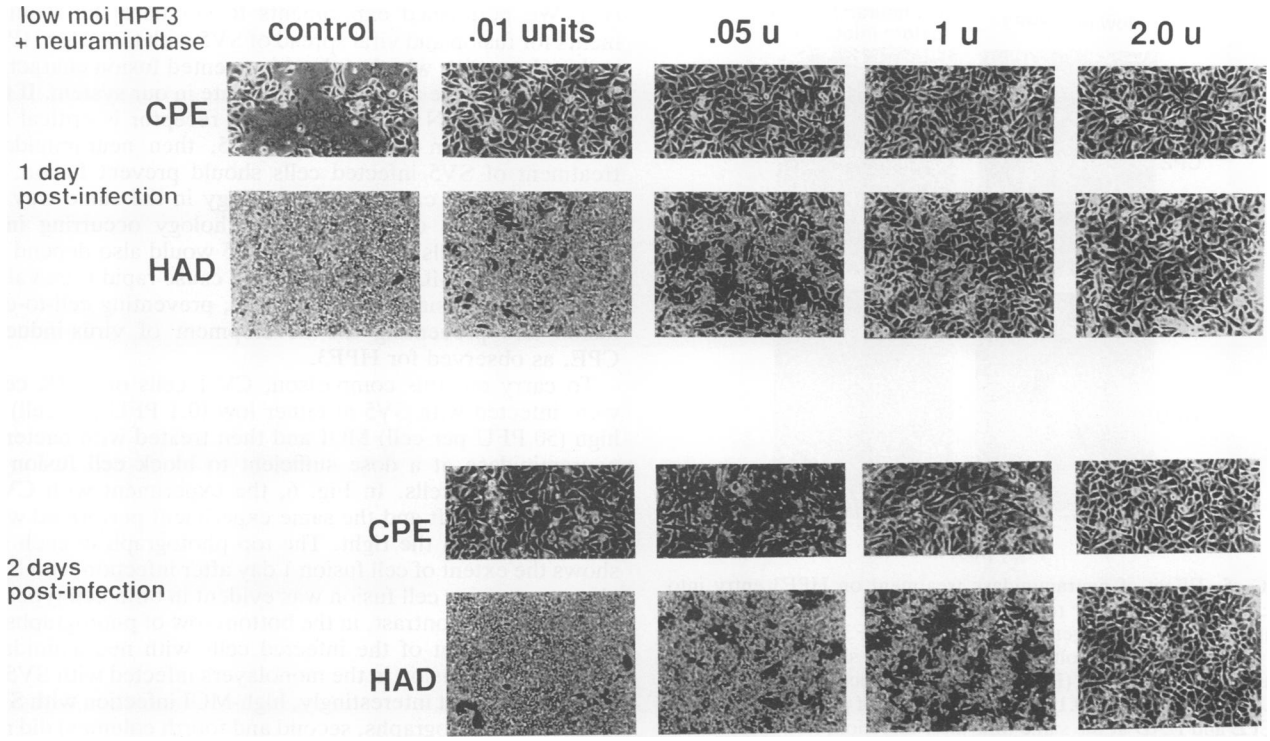

FIG. 4. Effect of treatment with various levels of exogenous neuraminidase on HPF3-mediated cytopathic effects and viral spread: sialic acid requirements for spread of infection and HPF3-mediated cell fusion. Monolayer cultures of CV-1 cells were infected with HPF3 at a low MOI (0.01 PFU per cell). The first column of photographs shows the CPE and degree of hemadsorption (HAD) at 1 day after infection, after addition of HPF3 alone at a low MOI. The second column of photographs shows the CPE and HAD at 1 day (top) and 2 days (bottom) after infection; however, in this experiment 0.01 U of *C. perfringens* neuraminidase was added after viral infection. The last three columns of photographs show the CPE and HAD of the neuraminidase-treated infected cells at the same times after infection (1 and 2 days), after progressively higher amounts of neuraminidase had been added. The amounts of exogenous neuraminidase added after low-MOI viral infection range from 0.01 U in the second column to 2.0 U in the fifth column. Progression of the infection can be monitored by observing the increase in HAD-positive cells from 1 day (top) to 2 days (bottom) after infection.

after treatment with either bacterial neuraminidase or UV-inactivated virus. Released sialic acid was assayed by HPLC, with a sensitivity of 2 pmol.

Bacterial neuraminidase released the following amounts of sialic acid residues from the treated cells: 0.01 U, 5.9 nmol; 0.1 U, 6.9 nmol; 1 U, 8.4 nmol; and 2 U, 8.5 nmol. Predictably, the more neuraminidase was used, the more sialic acid was released. At the amount of bacterial neuraminidase sufficient to block fusion in the experiment shown in Fig. 2 (0.1 U/3 $\times$ 10^6 cells), 6.9 nmol of sialic acid had been released.

We assayed the amount of sialic acid released by UV-inactivated virus, using the same amount of virus that blocks fusion in a low-MOI infection (10^8 PFU [number of cells, 3 $\times$ 10^6]). With this amount of UV-inactivated virus, 303 pmol of sialic acid was released. This release of sialic acid by UV-inactivated virus amounts to 4.4% of that released by 0.1 U of bacterial neuraminidase, so we determined whether we could increase the amount of sialic acid released by increasing the amount of UV-inactivated virus and the number of cells. With 6 $\times$ 10^8 PFU of UV-irradiated virus (number of cells, 10^7), 16 nmol of sialic acid was released. The amount of sialic acid released under these conditions was in the range of the amount released by bacterial neuraminidase.

Effect of treatment with various levels of exogenous neuraminidase on viral spread: sialic acid receptor requirements for spread of infection and HPF3-mediated cell fusion. The finding that bacterial neuraminidase treatment of cells infected at a low MOI allowed spread of the infection without cell fusion suggested that there are different sialic acid receptor requirements for a virus to infect a cell, by fusion of the viral envelope with the plasma membrane, than for fusion between cells. To test this hypothesis, CV-1 cells were infected with HPF3 at a low MOI and then treated with several different amounts of bacterial neuraminidase. The cells were examined for the presence of syncytia and assayed for the presence of virus infection by hemadsorption assays. The results are shown in Fig. 4. The first column of photographs demonstrates extensive cell fusion 24 h after infection with a low MOI of HPF3 alone, and all of the cells in the monolayer were hemadsorption positive; each of the next columns of photographs in Fig. 4 shows the CPE and degree of hemadsorption at the same times after infection, after progressively higher amounts of neuraminidase had been added. At the lowest dose of neuraminidase tested, 0.01 U, only a few foci of fused cells were present in the entire culture dish, but most of the cells were infected. When the dosage of neuraminidase was increased, there was no fusion at all between cells in any of the cultures. Infection still spread through the culture, as indicated by positive hemadsorption; however, the percentage of infected cells decreased as the amount of neuraminidase increased. When these cultures were incubated longer, the viral infection continued to spread in the absence of cell fusion. At the

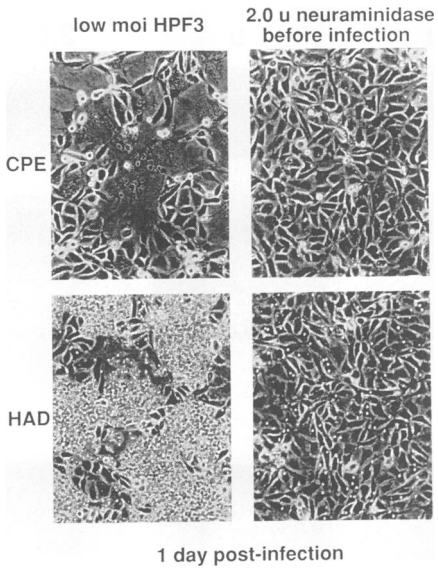

FIG. 5. Effect of neuraminidase treatment on HPF3 entry into cells: sialic acid receptor requirements for HPF3 entry. Monolayer cultures of CV-1 cells were infected with HPF3 at an MOI of 0.1 PFU per cell. The left column of photographs shows the CPE and degree of hemadsorption (HAD) 1 day after infection, after infection with HPF3 at a MOI of 0.1. The right column of photographs shows the CPE and HAD at the same time after infection; however, in this experiment the monolayers of CV-1 cells were treated with 2.0 U of *C. perfringens* neuraminidase before infection with HPF3 and neuraminidase was continuously present in the medium.

highest level of neuraminidase treatment (2.0 U), the viral infection did not spread. Although at a level of neuraminidase treatment of ≤1.0 U sufficient sialic acid receptors remain for virus to infect a cell but not enough to allow cell fusion, at a level of neuraminidase treatment of 2.0 U the remaining sialic acid receptors are insufficient even for virus infection.

Effect of neuraminidase treatment on viral entry: sialic acid receptor requirements for viral entry. The fact that treatment of HPF3-infected cells with 2.0 U of neuraminidase prevented cell-to-cell spread of virus suggested that this level of neuraminidase treatment should prevent initial viral entry into uninfected cells. To test this, cells were treated with 2.0 U of neuraminidase and then infected with HPF3 at an MOI of 0.1 PFU per cell. Neuraminidase was continuously present in the medium after exposure to the virus in order to prevent the cells from regenerating surface sialic acid. Figure 5 shows that, as expected, no viral infection was detected in cells which had been treated with 2.0 U of neuraminidase. The hemadsorption assay readily detected infected cells in the control experiments in which no exogenous neuraminidase was present (Fig. 5, first column of photographs) and in experiments in which lower levels of neuraminidase were present (data not shown). The degree of cell surface desialation sufficient to abolish viral spread after infection is therefore also sufficient to block initial viral entry.

Effect of low-level exogenous neuraminidase on CPE produced by SV5. In contrast to the data for HPF3 and several other paramyxoviruses suggesting that both HN and F are involved in fusion, it has been demonstrated by expression of the SV5 F protein gene in CV-1 cells that the SV5 F protein can mediate membrane fusion in the absence of HN (15). We performed experiments to compare the requirements for fusion and viral spread of SV5 with those for HPF3 and to determine whether the documented fusion characteristics of SV5 have a biological correlate in our system. If the interaction of HN with a sialic acid receptor is critical for membrane fusion mediated by SV5, then neuraminidase treatment of SV5 infected cells should prevent fusion. In addition, if SV5 causes cytopathology in the same way as HPF3, then the degree of cytopathology occurring in a monolayer of cells infected with SV5 would also depend on the MOI. High-MOI infection would cause rapid removal of the cell surface sialic acid receptors, preventing cell-to-cell fusion and preventing the development of virus-induced CPE, as observed for HPF3.

To carry out this comparison, CV-1 cells or BHK cells were infected with SV5 at either low (0.1 PFU per cell) or high (50 PFU per cell) MOI and then treated with bacterial neuraminidase at a dose sufficient to block cell fusion in HPF3-infected cells. In Fig. 6, the experiment with CV-1 cells is on the left and the same experiment performed with BHK cells is on the right. The top photograph in each set shows the extent of cell fusion 1 day after infection with SV5 alone; extensive cell fusion was evident in both cell types at both MOIs. In contrast, in the bottom row of photographs in Fig. 6, treatment of the infected cells with neuraminidase prevented cell fusion in the monolayers infected with SV5 at a low MOI. Most interestingly, high-MOI infection with SV5 (Fig. 6, top photographs, second and fourth columns) did not prevent the development of CPE, and treatment of these high-MOI infected cells with neuraminidase had no effect on the degree of CPE (Fig. 6, bottom photographs, second and fourth columns).

DISCUSSION

Infection of cultured CV-1 cells with HPF3 at a low MOI (≤1 PFU per cell) leads to cell fusion and results in the death of the cells in the culture. When cells are infected with the same virus at a higher MOI (≥5 PFU per cell), the infected cells manifest little evidence of viral infection, remain totally free of syncytia, and yield PI cells that support the continuous replication of the virus (11, 12). We reported that the failure of cells PI with HPF3 to fuse with each other was due to the lack of a sialic acid-containing receptor for the viral HN glycoprotein on their surface, implicating the HN glycoprotein in membrane fusion caused by the virus (13). The experiments described in the present paper show that high-MOI infection prevents cell-cell fusion by the presence of high levels of neuraminidase in the viral inoculum. A normally fusogenic low-MOI infection is transformed into a nonfusogenic infection by the addition of neuraminidase, either as purified enzyme or in the form of UV-irradiated virus. The high levels of neuraminidase present in the high-MOI inoculum result in removal of the cell surface sialic acid receptors, preventing cell-to-cell fusion and thus preventing the development of CPE.

Treatment with UV-inactivated virus blocked fusion even when it removed much less sialic acid than bacterial neuraminidase. We conjecture that the sialic acid linkages cleaved by the HPF3 neuraminidase are more limited and specific than those cleaved by bacterial neuraminidase; the HPF3 enzyme removes only those residues involved in the interaction of parainfluenza virus with the cell, while the majority of the residues cleaved by bacterial neuraminidase may be irrelevant to the virus. This would explain why, in order to block fusion, the amount of sialic acid released by

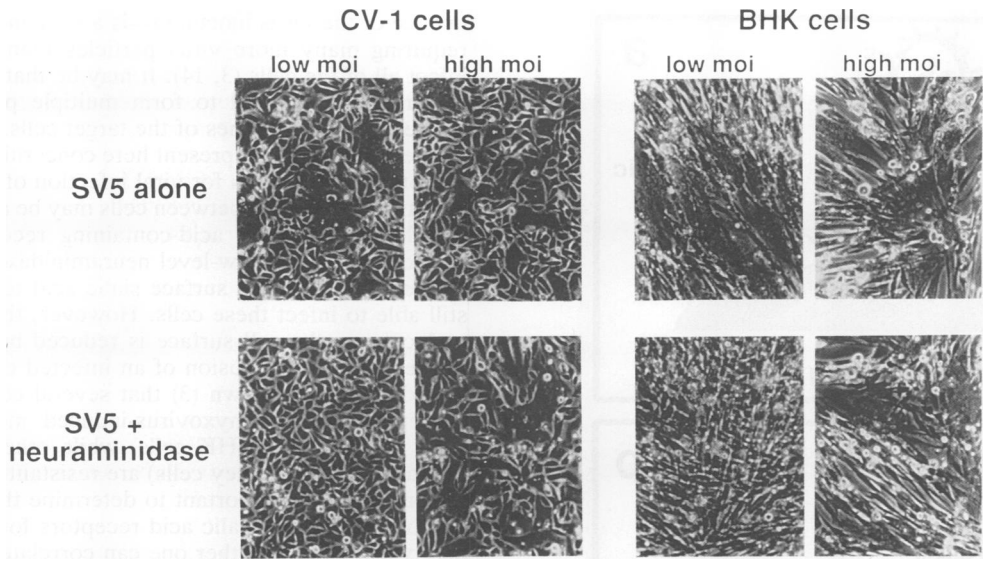

FIG. 6. Effect of low-level exogenous bacterial neuraminidase on CPE produced by infection with SV5 at low and high MOIs. Monolayer cultures of CV-1 cells (left) or BHK cells (right) were infected with either a low (0.1 PFU per cell) or high (50 PFU per cell) MOI of SV5. The top photographs in each set show the extent of CPE after infection with SV5 alone. The photographs were taken at 30 h after infection for the CV-1 cells and at 24 h after infection for the BHK cells. The bottom photographs of each set show the CPE at the same times after infection; however, in this experiment 0.1 U of *C. perfringens* neuraminidase was added after viral infection.

UV-inactivated virus can be so low relative to that released by bacterial neuraminidase; the only residues removed are those actually utilized by the virus. In addition, it is likely that the UV-inactivated virus glycoproteins are incorporated into the plasma membrane of the cell and remain active from that location. The decrease in cell surface sialic acid receptors, initially caused by the virus in the inoculum, may then be an ongoing process maintained by the appearance of newly synthesized HN molecules in the plasma membrane of the infected cell. The progeny HN glycoprotein molecules may serve to continuously remove sialic acid receptors from the cell surface, so that the infected cells are unable to regenerate their normal array of surface sialic acid. These results offer an explanation for the consistent development of persistence in cells infected at a high MOI; prevention of cell-to-cell fusion prevents cell death and affords the opportunity for the development of persistent infection.

Our studies of cells PI with HPF3 showed that the HN protein is essential for the fusion process in cells infected by this virus, and evidence from studies of several other paramyxoviruses (2, 5, 6, 8–10, 16, 18–23) has revealed that both the HN and the F protein participate in the fusion process. It has been demonstrated that the SV5 F protein can mediate membrane fusion in the absence of HN and that coexpression of F and HN has little effect on the degree of syncytium formation (5, 15). In addition, modification of the SV5 F protein by site-directed mutagenesis resulted in increased fusogenicity (4), confirming the ability of the SV5 F protein to function independently of HN in mediating fusion. Evidence for the sufficiency of the SV5 F protein in membrane fusion during natural viral infection has been provided by the demonstration that an anti-HN antiserum was not able to prevent spread of SV5 by cell-cell fusion in cell culture (7). Because of these reported differences in the function of HPF3 and SV5 F and HN in expression experiments and during viral infection, it was important to compare the requirements in our experimental system for fusion and viral spread of SV5 with those for HPF3.

Treatment of SV5-infected cells with neuraminidase prevented cell fusion in the monolayers infected at a low MOI, in agreement with our findings for HPF3. However, in marked contrast with HPF3, high-MOI infection with SV5 did not prevent the development of CPE and treatment of these cells infected with SV5 at a high MOI with neuraminidase had no effect on the degree of CPE. These results show that although at a low MOI the pathogeneses of HPF3 and SV5 are similar, in a high-MOI infection the manifestation of infection with each virus is strikingly different. The fact that the fusion behaviors of HPF3 and SV5 are different at a high MOI supports the differences in observed fusogenic activity of the expressed F glycoproteins of these viruses.

Our finding that neuraminidase treatment of HPF3-infected cells can prevent cell-cell fusion strengthens our previous suggestion that HN's interaction with its receptor is critical to fusion mediated by the F protein. Although it is possible that physical interaction between F and HN occurs in the plane of the infected cell membrane, this interaction cannot be the sole function of HN in the fusion process; both glycoproteins are present in the same membrane of cells PI with HPF3, yet fusion does not occur unless HN on an infected cell interacts with sialic acid receptors on the target cell membrane (13). In one potential explanation, HN may hold the target cell plasma membrane in the requisite spatial configuration for F to mediate fusion. The finding that only HN and F from the same parainfluenza virus type can cooperate in coexpression experiments to produce cell fusion (6) is consistent with this explanation; HN may need to interact simultaneously with the target cell receptor and with the F protein of the same parainfluenza virus type. Alternatively, there may be specific spatial requirements for F to mediate fusion, and only the type-matched HN can provide the correct bridging distance between F and the target cell membrane.

The finding that HPF3 virions are able to infect cells that have been neuraminidase treated and yet these infected cells are not functional in fusing with uninfected cells implies that

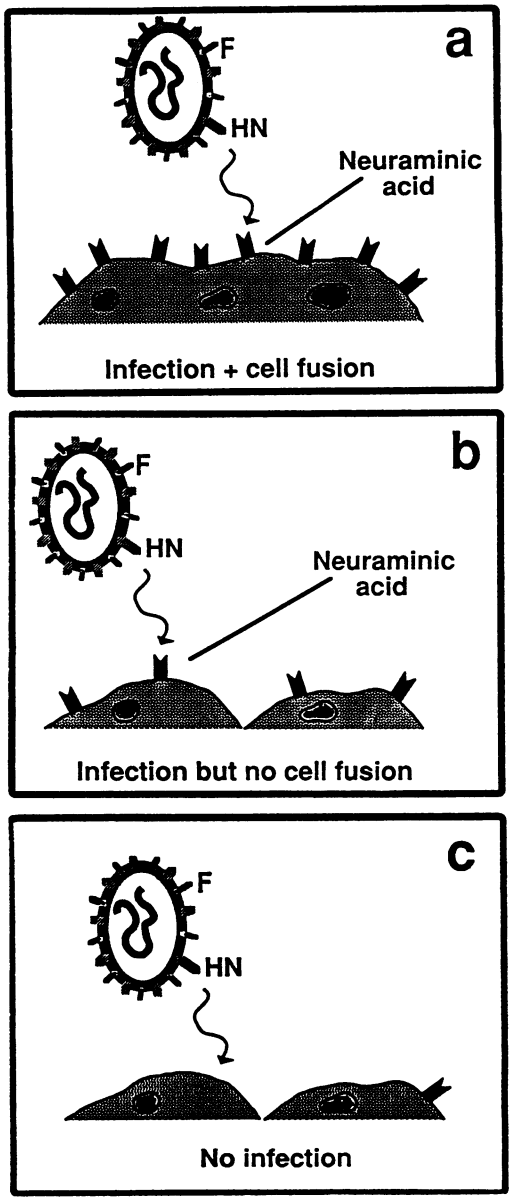

FIG. 7. A schematic diagram of the interactions of HPF3 with cells that contain various levels of virus receptor molecules and the outcomes of each type of interaction. For details, see text.

there are different neuraminic acid requirements for a virus particle to infect a cell by fusion of the viral envelope with the plasma membrane than for fusion of an infected cell with an uninfected cell. There are several possible explanations for this finding. The configuration of the glycoproteins on the surface of a virus particle may differ from that on the surface of an infected cell, and only a subset of the glycoproteins on the surface of the cell may be in a form capable of mediating fusion. Another possibility is that in order for fusion of two cell membranes to occur, more points of contact between HN and F on the infected cell surface and receptors on an uninfected cell are needed than for fusion of a viral envelope with a much smaller portion of a cell's membrane. Consistent with this hypothesis is the fact that so-called "fusion-from-without"—cell-to-cell fusion as an immediate consequence of the virus inoculum—is a very inefficient process, requiring many more virus particles than are required to infect all of the cells (3, 14). It may be that such high levels of virus are required to form multiple points of contact between the membranes of the target cells.

The results that we present here concerning the sialic acid receptor requirements for viral infection of a cell compared with those for fusion between cells may be a consequence of the density of sialic acid-containing receptors on a cell surface. Relatively low-level neuraminidase treatment does not remove all of the surface sialic acid receptors; virus is still able to infect these cells. However, the density of this molecule on the cell surface is reduced below a threshold needed for efficient fusion of an infected cell with an uninfected one. It is known (3) that several cell lines are very susceptible to paramyxovirus-induced membrane fusion, such as CV-1 and BHK cells, while other lines (MDBK, primary monkey kidney cells) are resistant to virus-induced fusion. It will be important to determine the relative densities of cell surface sialic acid receptors for HPF3 on these cell types to see whether one can correlate the fusibility of the cells with the density of this receptor.

The data presented here suggest an explanation for the development of persistent infection after high-MOI infection with HPF3. In the schematic diagram shown in Fig. 7, panel a represents the events during an acute, low-MOI infection with HPF3; virus spreads and mediates cell-cell fusion. Fig. 7c represents neuraminidase treatment removing virtually all of the cell surface sialic acid receptors; this blocks viral infection. Fig. 7b depicts what we believe occurs during a high-MOI infection. A sufficient number of sialic acid receptors are removed by the viral neuraminidase in the inoculum to prevent cell fusion but still allow viral infection and spread. Prevention of cell-to-cell fusion by the neuraminidase present in the inoculum thus affords the opportunity for the development of persistent infection. Experiments to explore further the factors which contribute to establishment and maintenance of persistent infection are currently under way.

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REFERENCES

1. **Choppin, P. W., and A. Scheid.** 1980. The role of viral glycoproteins in adsorption, penetration, and pathogenicity of viruses. Rev. Infect. Dis. **2**:40–61.
2. **Ebata, S. N., M.-J. Cote, C. Y. Kang, and K. Dimock.** 1991. The fusion and hemagglutinin-neuraminidase glycoproteins of human parainfluenza virus 3 are both required for fusion. Virology **183**:437–441.
3. **Holmes, K. V., and P. W. Choppin.** 1966. On the role of the response of the cell membrane in determining virus virulence. Contrasting effects of the parainfluenza virus SV5 in two cell types. J. Exp. Med. **124**:501–520.
4. **Horvath, C. M., and R. A. Lamb.** 1992. Studies on the fusion peptide of a paramyxovirus fusion glycoprotein: roles of conserved residues in cell fusion. J. Virol. **66**:2443–2455.
5. **Horvath, C. M., R. G. Paterson, M. A. Shaughnessy, R. Wood, and R. A. Lamb.** 1992. Biological activity of paramyxovirus fusion proteins: factors influencing formation of syncytia. J. Virol. **66**:4564–4569.

6. **Hu, X., R. Ray, and R. W. Compans.** 1992. Functional interactions between the fusion protein and hemagglutinin-neuraminidase of human parainfluenza viruses. J. Virol. **66**:1528–1534.
7. **Merz, D. C., A. Scheid, and P. W. Choppin.** 1979. Importance of antibodies to the fusion glycoprotein of paramyxoviruses in the prevention of spread of infection. J. Exp. Med. **151**:275–288.
8. **Merz, D. C., and J. S. Wolinsky.** 1981. Biochemical features of mumps virus neuraminidases and their relationships with pathogenicity. Virology **114**:218–227.
9. **Merz, D. C., and J. S. Wolinsky.** 1983. Conversion of nonfusing mumps virus infections by selective proteolysis of the HN glycoprotein. Virology **131**:328–340.
10. **Morrison, T., C. McQuain, and L. McGinnes.** 1991. Complementation between avirulent Newcastle disease virus and a fusion protein gene expressed from a retrovirus vector: requirements for membrane fusion. J. Virol. **65**:813–822.
11. **Moscona, A.** 1991. Defective interfering particles of human parainfluenza virus type 3 are associated with persistent infection in cell culture. Virology **183**:821–824.
12. **Moscona, A., and M. S. Galinski.** 1990. Characterization of human parainfluenza virus type 3 persistent infection in cell culture. J. Virol. **64**:3212–3218.
13. **Moscona, A., and R. W. Peluso.** 1991. Fusion properties of cells persistently infected with human parainfluenza virus type 3: participation of hemagglutinin-neuraminidase in membrane fusion. J. Virol. **65**:2773–2777.
14. **Okada, Y.** 1962. Analysis of giant polynuclear cell formation caused by HVJ virus from Ehrlich's ascites tumor cells. I. Microscopic observation of giant polynuclear cell formation. Exp. Cell Res. **26**:98–107.
15. **Paterson, R. G., S. W. Hiebert, and R. A. Lamb.** 1985. Expression at the cell surface of biologically active fusion and hemagglutinin/neuraminidase proteins of the paramyxovirus simian virus 5 from cloned cDNA. Proc. Natl. Acad. Sci. USA **82**:7520–7524.
16. **Portner, A., R. A. Scroggs, and D. W. Metzger.** 1987. Distinct functions of antigenic sites of the HN glycoprotein of Sendai virus. Virology **158**:61–68.
17. **Powell, L. D., and G. W. Hart.** 1986. Quantitation of picomole levels of N-acetyl and N-glycolylneuraminic acids by a HPLC-adaptation of the thiobarbituric acid assay. Anal. Biochem. **157**:179–185.
18. **Sakai, Y., and H. Shibuta.** 1989. Syncytium formation by recombinant vaccinia viruses carrying bovine parainfluenza 3 virus envelope protein genes. J. Virol. **63**:3661–3668.
19. **Shibuta, H., T. Kanda, A. Hazama, A. Adachi, and M. Matumoto.** 1981. Parainfluenza 3 virus: plaque-type variants lacking neuraminidase activity. Infect. Immun. **34**:262–267.
20. **Shibuta, H., A. Nozawa, T. Shioda, and T. Kanda.** 1983. Neuraminidase activity and syncytial formation in variants of parainfluenza 3 virus. Infect. Immun. **41**:780–788.
21. **Tsurudome, M., A. Yamada, M. Hishiyama, and Y. Ito.** 1986. Monoclonal antibodies against the glycoproteins of mumps virus: fusion inhibition by anti-HN monoclonal antibody. J. Gen. Virol. **67**:2259–2265.
22. **Waxham, M. N., and J. Aronowski.** 1988. Identification of amino acids involved in the sialidase activity of the mumps virus hemagglutinin-neuraminidase protein. Virology **167**:226–232.
23. **Waxham, M. N., and J. S. Wolinsky.** 1986. A fusing mumps virus variant selected from a nonfusing parent with the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid. Virology **151**:286–295.
24. **Wechsler, S. L., D. M. Lambert, M. S. Galinski, M. A. Mink, O. Rochovansky, and M. Pons.** 1987. Immediate persistent infection by human parainfluenza virus 3: unique fusion properties of the persistently infected cells. J. Gen. Virol. **68**:1737–1748.