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Characterization of a Recombinant Herpes Simplex Virus 1 Designed To Enter Cells via the IL13Rα2 Receptor of Malignant Glioma Cells

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Malignant glioma tumor cells in situ exhibit on their surfaces the interleukin 13 (IL-13) receptor designated IL13Rα2. To target herpes simplex virus 1 to this receptor, we constructed a recombinant virus (R5111) in which the known heparan sulfate binding sites in glycoproteins B and C were deleted and IL-13 was inserted into both glycoproteins C and D. We also transduced a baby hamster kidney cell line lacking the known viral receptors (J1-1) and Vero cells with a plasmid encoding IL13Rα2. The J1-1 derivative (J-13R) cell line is susceptible to and replicates the R5111 recombinant virus but not the wild-type parent virus. We report the following. (i) Expression of IL13Rα2 was rapidly lost from the surface of transduced cells grown in culture. The loss appeared to be related to ligands present in fetal bovine serum in the medium. None of the malignant glioma cell lines cultivated in vitro and tested to date exhibited the IL13Rα2 receptor. (ii) Soluble IL-13 but not IL-4 or IL-2 blocked the replication of R5111 recombinant virus in J-13R cells. (iii) The endocytosis inhibitor PD98059 blocked the replication in J1-1 cells of a mutant lacking glycoprotein D ($gD^{-/-}$) but not the replication of R5111 in the J-13R cells. We conclude that R5111 enters cells via its interaction with the IL13Rα2 receptor in a manner that cannot be differentiated from the interaction of wild-type virus with its receptors.

The experiments described in this report stemmed from two considerations. In the last decade, several laboratories have attempted to develop herpes simplex virus 1(HSV-1) mutants suitable for cytoreductive therapy of human malignant gliomas. It is estimated that these incurable tumors claim 10,000 lives per year in the United States alone, and current therapeutic modalities seldom produce long-lasting remissions (14). Of the various mutants tested in the mouse model, two such mutants found their way to clinical trials. Both mutants lack the $\gamma_1$34.5 genes shown earlier to be essential for viral replication in the central nervous system in experimental animal systems (15, 21). One mutant, G207, lacked in addition the U_L39 gene encoding the large subunit of ribonucleotide reductase (15, 16). While both mutants exhibited a satisfactory safety profile within the range of concentrations injected into the tumor mass, neither mutant exhibited unambiguous therapeutic potential as tested. The safety of the $\gamma_1$34.5 null mutant hinges on the fact that it is unable to block the shutoff of protein synthesis resulting from the activation of protein kinase R (3, 12). In consequence, viral replication is reduced and the mutant is unable to spread from cell to cell. One potential counterindication for its use is the emergence of second-site mutations that block the activation of protein kinase R and show enhanced capacity to replicate in the central nervous system (2, 11, 18). The introduction of a second deletion reduced the risk of second-site, complementary mutation that partially restored virulence. However, the double mutant is further debilitated and replicates only in dividing cells. In malignant gliomas only a fraction of the tumor cells actively divide, and hence, the double-deletion mutant would be predicted to have a limited cytocidal activity. One obvious solution is to develop mutants that retain the capacity to replicate and spread from cell to cell but that specifically target malignant gliomas.

HSV-1 contains in its envelope two glycoproteins, B and C (gB and gC), which interact with heparan sulfate on the surfaces of cells, and one glycoprotein, gD, which initiates the process of entry into cells through its interactions with either one of two protein receptors, HveA, a member of the tumor necrosis factor alpha receptor family, or nectin1, a member of a family of proteins that bridge the surfaces of adjacent cells (4, 9, 19). To construct a recombinant virus with enhanced specificity for malignant gliomas, we deleted the heparan sulfate binding site in gB, replaced the amino-terminal domain including at least a portion of the heparan sulfate binding site of gC with interleukin 13 (IL-13), and inserted IL-13 into the amino-terminal domain of gD (Fig. 1). The insertion of IL-13 was based on the evidence that malignant glioma tumor cells contain on their surfaces a receptor for IL-13 (IL13Rα2) that differs from the more common IL-13 receptor in several important characteristics. Thus, the IL13Rα2 is found, in addition to grade IV malignant gliomas, in testes but not in other organs (6, 7, 17). It binds IL-13 but not IL-4, it is a monomer, and it has a short cytoplasmic domain that does not signal (5, 6). The virus carrying the mutations described above and designated R5111 enters and replicates in baby hamster kidney (BHK) cell lines ectopically expressing IL13Rα2 but lacking HveA or nectin1, whereas the wild-type parent virus, HSV-1(F), does not (24). Extensive tests have now shown that R5111 retains the capacity to enter and replicate in cells exhibiting only HveA or nectin1 receptor. In J-13R cells, R5111 is not able to avail itself of the HveA or nectin1 binding sites. The central question, therefore, is the mechanism by which R5111

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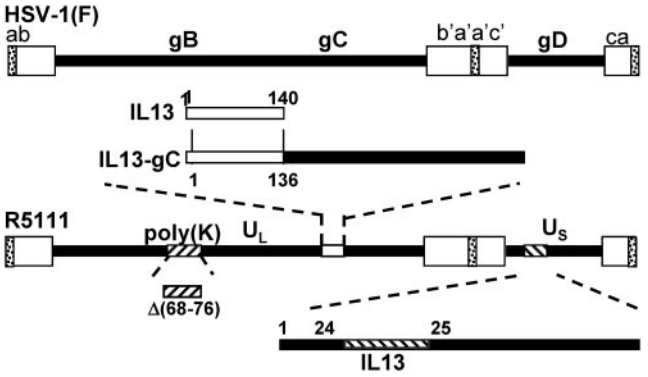

FIG. 1. Schematic representation of the structure of the R5111 recombinant virus. The construction of the virus as published elsewhere (24).

recombinant virus enters the J-13R cells, whether its entry is dependent entirely on the IL13Rα2 and whether it enters by endocytosis or by the more conventional method of fusion of the envelope with the plasma membrane.

In this report we show that the ectopic expression of IL13Rα2 in a transduced baby hamster kidney cell line (J1-1) or Vero cells is unstable, especially at high serum concentrations, that entry is blocked by exposure of the cells to IL-13 but not to IL-4 or IL-2, and that an inhibitor of endocytosis active in the J1-1 cell lines has no effect on the entry of R5111 recombinant virus into J-13R cells. We should note that this is the first report of a recombinant HSV-1 mutant that interacts with a novel receptor in a manner indistinguishable from the interaction of the virus with a natural receptor.

MATERIALS AND METHODS

Cells and Viruses. Vero cells were obtained from American Type Culture Collection (Rockville, MD) and maintained in Dulbecco's modification of Eagle minimal essential medium (DMEM) supplemented with 10% fetal bovine serum. J1-1, a derivative of a BHK thymidine kinase-minus cell line, lacks both HveA and nectin 1 HSV-1 receptors (4). The J-HveA and J-Nectin cell lines, J1-1 derivatives expressing HveA and nectin, respectively, were kind gifts from Campadelli-Fiume (University of Bologna, Italy).

Human malignant glioma cells U-373 and D-37 were obtained from Y. Gillespie (University of Alabama) and grown in DMEM supplemented with 10% fetal bovine serum. The human glioma cell line U-87 and the pancreatic cancer cell line PaCa were kindly provided by R. Weichselbaum (The University of Chicago) and cultured in DMEM supplemented with 10% fetal bovine serum.

The construction of R5111 recombinant virus was described elsewhere (24). As illustrated in Fig. 1, the heparan sulfate binding site in gC was degraded by the replacement of amino acids 1 to 136 with the first 140 amino acids of IL-13, whereas the binding site in gB (polylysine tract) was deleted. In addition, the IL-13 residues 1 to 140 were inserted between amino acids 24 and 25 of gD to target the virus to the IL13Rα2 receptor.

The $gD^{-/-}$ mutant lacks both the gD gene and the gD protein in its envelope. It is produced by infection of cells lacking the complementing gD gene with a mutant virus stock ($gD^{-/+}$) produced in complementing cells. The procedure for the production and titration of $gD^{-/-}$ virus was described elsewhere (25). $gD^{-/-}$ virus enters cells by endocytosis, and at high multiplicities in the absence of complementing gD, it causes cells to undergo apoptosis (25).

Antibodies. Monoclonal antibody against IL13Rα2 was purchased from Santa Cruz Biotechnology.

Reagents. The endocytosis inhibitor PD98059 was purchased from Cell Signaling Technology, Beverly, MA. IL-13, IL-4, and IL-2 were purchased from Research Diagnostics, Inc., Flanders, NJ.

Construction of cell line expressing IL13Rα2 receptor. The ILR13Rα2 open reading frame was inserted into the pcDNA 3.1 vector (Invitrogen; Carlsbad, CA) via the NheI and HindIII restriction endonucleases to generate the transfer plasmid pRB13-R2-1. The construct was sequenced to ensure fidelity. J1-1 cells were stably transfected with pRB13-R2-1 by using a Lipofectamine kit (Gibco-BRL) to generate J-13R cells which were selected on the basis of their resistance to neomycin (G418) (Invitrogen). G418-resistant clones were checked for expression of IL13Ra2 with the monoclonal antibody to the protein. The same protocol was used to generate Vero-13R cells.

Cell surface immunostaining. G418-resistant cells in 75-cm^2 flasks were harvested and reacted with the anti-IL13Rα2 antibody (1:500) for 15 min on wet ice. The cells were then rinsed twice with cold phosphate-buffered saline and then reacted for 15 min with a 1:64 dilution of goat anti-mouse immunoglobulin G conjugated to fluorescein isothiocyanate (FITC) (Sigma, St. Louis, Mo.) in ice-cold phosphate-buffered saline. After two rinses the cells were suspended in 1 ml of cold medium containing 5% fetal bovine serum and stored on wet ice in a 12- by 75-mm Falcon (Becton-Dickinson) polypropylene tube for further fractionation in a cell sorter or for immunofluorescence analyses.

Fluorescence-activated cell sorting. Flow cytometry was performed on a FACScalibur (Becton-Dickinson, Mountain View, CA) cell sorter. Approximately 5×10^4 purified FITC-positive cells were isolated and seeded on a six-well plate for amplification at 37°C.

Immunofluorescence analyses. Ten μl of the surface-stained cells were plated onto four-well glass slides and mounted in 90% glycerol. Slides were analyzed with the aid of a Zeiss confocal fluorescence microscope. A total of 200 cells in adjacent fields were examined for the presence of surface fluorescence.

Virus titration. Replicate cultures of J-13R and Ver-13R cells were exposed to 0.1 PFU of R5111 or HSV-1(F) virus per cell. After 24 h of incubation, the cells were harvested and disrupted by sonication. Viral progeny was titrated on Vero cells.

RESULTS

Properties and expression of the IL13Rα2 receptor in J-13R and Vero-13R cells. In the course of the initial series of experiments, we noted that the expression of the IL13Rα2 receptor transduced into the J1-1 cells was unstable. The fraction of transduced cells expressing the IL13Rα2 receptor as measured with the aid of the cell sorter was 3 to 5% of the initial population (Fig. 2). After sequential sorting followed by single passages, the percentage of positive cells climbed to 96 percent (Fig. 3). The number of positive cells expressing the IL13Ra2 receptor, determined by surface immunostaining of unfixed cells, remained relatively constant in three subsequent passages, and the expanded population was frozen. However, the thawed stock yielded only 60% positive cells, and the percentage of positive cells declined on serial passage.

One possible explanation for the observed results was that the cultivation of cells in medium containing fetal bovine serum resulted in the selection of cells lacking the receptor. To test this hypothesis, the cells were maintained for one passage in media containing from 0 or 5% to 20% fetal bovine serum. As illustrated in Table 1, the fraction of J-13R cells exhibiting the IL13Rα2 receptor on their surfaces diminished dramatically in a single passage, from 61% to as little as 7% in medium containing 20% fetal bovine serum. The fraction of positive Vero-13R cells decreased from 82% in passage 1 after thawing to 27% and 19%, respectively, after a single passage in media containing 10 and 20% fetal bovine serum, respectively. We conclude from these studies that in the presence of fetal bovine serum, either the IL13Rα2 receptor on cell surfaces was internalized and degraded or the serum blocked the growth of cells exhibiting the receptor. To discriminate between these hypotheses, replicate cultures of J-13R cells were exposed for 1 h to 1 ml of medium containing IL-13, IL-4, or IL-2 as described in footnote *a* of Table 2. At the end of the incubation period, the cultures were rinsed and reacted with anti-IL13Rα2 antibody

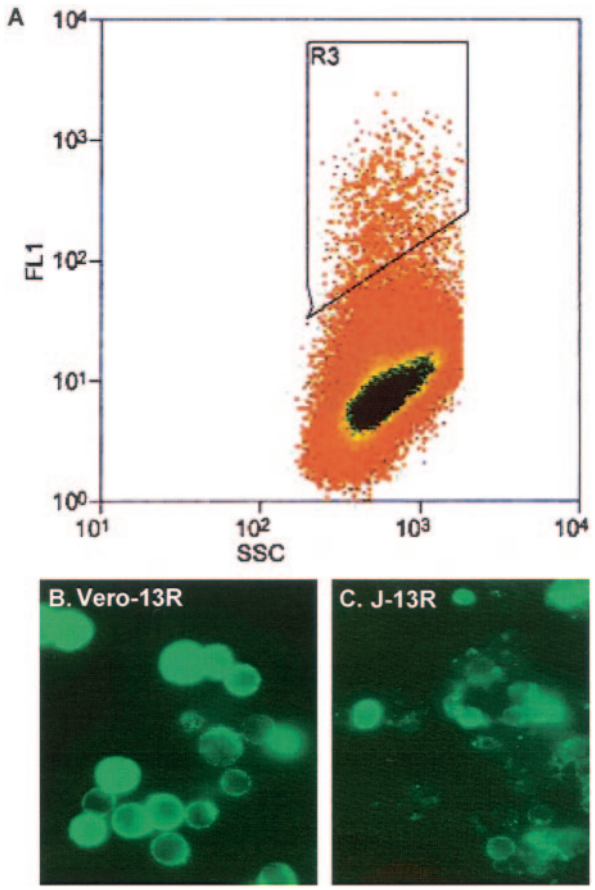

FIG. 2. Fluorescence-activated cell sorting of J-13R cells and surface fluorescence of J-13R and Vero-13R cells. Panel A. G418-resistant cells in 75-cm^2 flasks were removed and surface stained by FITC-conjugated anti-IL13Rα2 antibody as described in Materials and Methods. Flow cytometry was performed on a FACScalibur (Becton-Dickinson, Mountain View, CA). Panels B and C represent FITC-positive Vero-13R and J-13R cells, respectively. The FITC-positive cells, approximately 5×10^4 cells, comprising 3 to 5% of the total cell population, were collected and amplified.

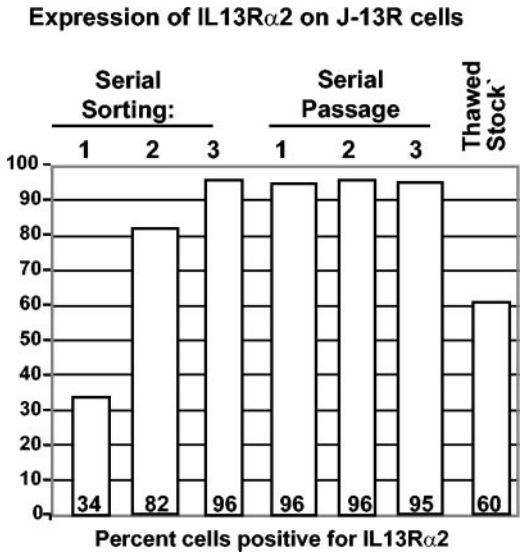

FIG. 3. Enrichment and stability testing for J-13R cells. The percentages of fluorescence-positive cells obtained after sequential sorting, three serial passages, and initial thawing of the frozen cell stock.

and examined with a Zeiss confocal microscope. In each preparation, 200 cells were counted in adjacent fields and the number of positive cells was recorded. The results shown in Table 2 indicate that IL13Rα2 disappeared from the surfaces of cells exposed to IL-13 but not from the surfaces of cells exposed to IL-4 or IL-2. The most likely explanation of these results, consistent with known behavior of surface receptors, is that the IL13Rα2–IL-13 complex was internalized and degraded.

Analyses for the presence of IL13Rα2 on the surface of malignant glioma cells grown in culture. The results described above raised the question of whether the IL13Rα2 receptor was unstable in J1-1 or Vero cells because these cells lacked the expression of genes characteristic of malignant glioma cells or whether the expression and stability of the IL13Rα2 receptor is incompatible with serial passage of cells in culture medium containing serum. To answer this question, we tested the PaCa, D-37, U-87, and U-373 cell lines for the presence of the IL13Rα2 receptor as described above. In these experiments, the J-13R cells served as a positive control. The results (data not shown) were that none of the serially passaged malignant glioma cell lines tested in these studies exhibited the IL13Rα2 receptor on their surface.

R5111 mutant retains capacity to infect cells exhibiting HveA or nectin1 receptor. On the basis of the site of insertion of IL-13 into the amino-terminal domain of gD, we predicted that IL-13 would retain the ability to infect cells via the nectin1 receptor but that the interaction with the HveA receptor would be degraded. To verify this prediction, we tested the ability of the R5111 mutant to replicate in J1-1 cells stably transduced with HveA, nectin1, or IL13Rα2 (J-HveA, J-Nectin, or J-13R), respectively. In these experiments, identical-size cultures were exposed to 0.1 PFU of HSV-1(F) or R5111 virus per cell. The cultures were harvested 24 h after infection, and titers of the virus were determined in Vero-IL-13 cells. The results, shown in Fig. 4, indicate that HSV-1(F) replicated in J-HveA and J-Nectin but not in J-13R cells. The amounts recovered from infected J-13R cells ($<10^2$ PFU) most likely represent residual

TABLE 1. Percent cells exhibiting IL13Rα2 receptor on cell surfaces as a function of serum concentration in the medium[a]

Cells or passage no.	Serum conc (%)	% Positive cells
J-13R		
1	5	61
2	0	51
2	5	42
2	10	31
2	20	7
Vero-13R cells		
1	5	82
2	5	76
2	10	27
2	20	29

[a] J-13R and Vero-13R cells from passage 1 after thawing were incubated in medium containing different concentrations of serum for 24 h. The cells were collected and surface stained by FITC-conjugated anti-IL13Rα2 antibodies as described in Materials and Methods. Positive cells were scored with the aid of a Zeiss confocal microscope. A total of 200 cells in adjacent fields were counted.

TABLE 2. Fraction of J-13R cells exhibiting surface IL13Rα2 receptor following exposure to IL-13, IL-4, or IL-2[a]

Treatment	% Positive
Mock	27
IL-13	0
IL-4	24
IL-2	27

[a] Replicate cultures of J-13R cells in medium containing 10% serum were either left untreated or exposed for 1 h to 10 ng of IL-13, IL-4, or IL-2, respectively. The cells were then rinsed, reacted with anti-IL13Rα2 antibody, and examined for surface fluorescence with the aid of a Zeiss confocal microscope. A total of 200 cells in adjacent fields were counted,

attached virus that has not penetrated into cells. R5111 virus replicated in all three cell lines, but its yield from J-13R cells was significantly lower than that obtained from the other two cell lines. One possible explanation for the lower yields is the loss of the IL13Rα2 receptor.

The entry of R5111 virus into J-13R cells is not dependent on endocytosis. In this series of experiments, J-13R cells were either mock treated or exposed to the endocytosis inhibitor PD98059 (30 μM, catalogue no. 9900; Cell Signaling Technology) (8, 10) for 1 h and then exposed to HSV-1(F) or R5111 recombinant virus (0.1 PFU/cell). The cells were harvested at 24 h after infection, and titers were determined on Vero-13R cells. As shown in Fig. 5, the J-13R cells infected with the wild-type parent virus yielded trace amounts of virus compatible with residues of virus attached to cell surfaces but which failed to penetrate. Cells infected with R5111 recombinant virus yielded approximately 10^4 more virus. Furthermore, the yields of virus obtained from J-13R cells exposed to R5111 recombinant virus in the presence of PD98059 were not significantly different from those obtained from untreated infected cells.

In light of the observation that PD98059 had no effect on R5111, the question arose whether the drug was effective in the J1-1 and derivative cell lines. To test the effectiveness of PD98059, J1-1 cells were transduced by exposure of the cells with baculoviruses encoding glycoprotein D. After 6 h, the cells were either left untreated or exposed for 1 h to 30 μM PD98059. The cells were then infected with 1 PFU of $gD^{-/-}$ mutant virus stock per cell. As reported earlier, $gD^{-/-}$ virus enters cells by endocytosis, and in cells lacking the gD genes, the virus causes apoptosis. The expectation was that the $gD^{-/-}$ virus would enter cells by endocytosis and that the gD produced in transduced cells would block cells from undergoing apoptosis and enable the synthesis of $gD^{-/+}$ virus. If PD98059 inhibitor is effective in blocking endocytosis, it would be expected that the yield of $gD^{-/+}$ virus would be significantly lower than that obtained in untreated transduced cells. As shown in Fig. 6, the amount of virus produced in untreated cells was 1,000-fold greater than that produced in treated cells.

We conclude from these studies that PD98059 inhibitor was effective in blocking endocytosis in J1-1 cells and that the R5111 mutant does not enter the J-13R cells by endocytosis.

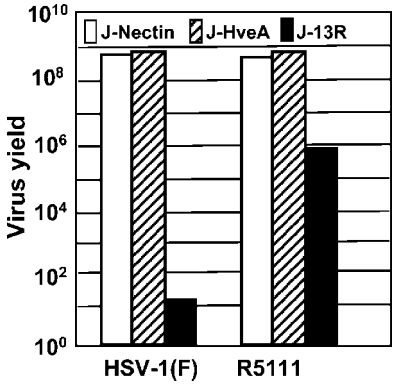

FIG. 4. Replication of R5111 recombinant in J-HveA, J-Nectin, and J-13R cell lines. J-HveA, J-Nectin, and J-13R cells were exposed to 0.1 PFU of R5111 recombinant virus or HSV-1(F) per cell and harvested 24 h after infection. Titers of progeny virus were determined on Vero-13R cells.

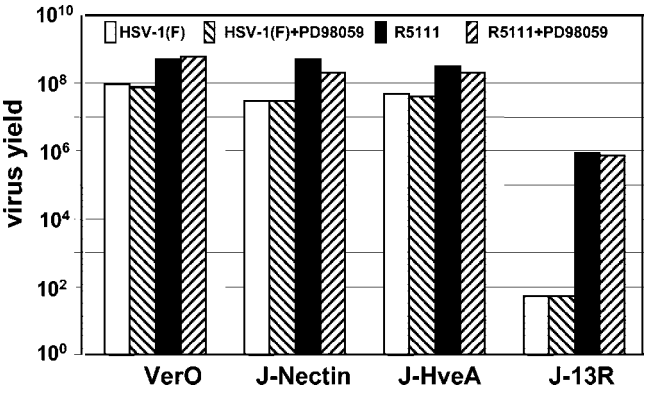

FIG. 5. PD98059, and inhibitor of endocytosis, does not block the replication of R5111 recombinant in J1-1 cells transduced with HveA, nectin1, or IL13Rα2. Vero, J-Nectin, J-HveA, or J-13R cells were exposed to 30 μM of PD98059 for 1 h. The cells were infected with 0.1 PFU of HSV-1(F) or R5111 recombinant virus per cell and then harvested 24 h after infection. Titers of progeny virus were determined on Vero-13R cells.

Infection and replication of R5111 recombinant virus in J-13R cells is blocked by IL-13. The objective of this series of experiments was to determine whether R5111 mutant virus enters cells via its interaction with IL13Rα2 or through some other, as yet unidentified receptor. To resolve this question, replicate cultures of J-13R or Vero-13R cells were mock treated or exposed for 1 h to 1 ng or 10 ng of IL-13, IL-4, or IL-2 per ml, respectively. The cells were then exposed to HSV-1(F) or R5111 (0.1 PFU/cell), and incubation at 37C was continued. The cells were harvested 24 h after infection. Progeny virus was titrated on untreated Vero-13R cells. The results, summarized in Fig. 7, were as follows.

IL-13 reduced the yield of R5111 from IL-13-treated, infected J-13R cells by approximately 1,000-fold compared with untreated infected J-13R cells. IL-4 or IL-2 had no effect on the replication of R5111 recombinant virus in the J-13R cells. As expected, the wild-type virus HSV-1(F) failed to replicate in J-13R cells. Exposure of the cells to the cytokines did not alter the lack of susceptibility of these cells to this virus.

DISCUSSION

The objective of the studies described in this report was to modify the structure of the wild-type virus HSV-1(F) in order to redirect the virus to a novel receptor. The receptor we have

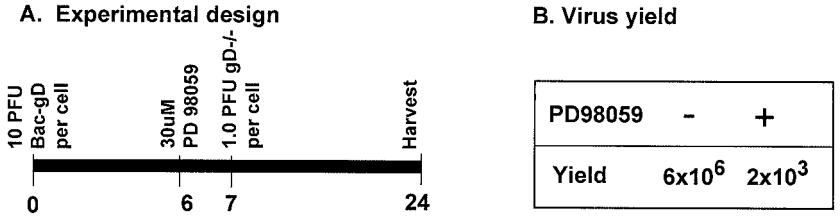

FIG. 6. PD98059 blocks the replication of $gD^{-/-}$ mutant virus in J1-1 cells transduced with gD. J1-1 cells were exposed for 6 h to 10 PFU of baculovirus encoding gD driven by an immediate-early human cytomegalovirus promoter and then for 1 h to 30 μM of PD98059. The cells were then infected with 1.0 PFU of $gD^{-/-}$ mutant virus stock per cell and harvested 24 h after infection (panel A). Titers of progeny virus were determined on Vero-13R cells (panel B).

selected is IL13Rα2, distributed in high-grade malignant gliomas, astrocytomas, and in normal testes (7, 17). In an earlier report, we showed that the recombinant virus carrying IL-13 in both gD and gC replicates in the J-13R cells, whereas the wild-type virus did not (24). A central question addressed in this report is the nature of the interaction of the R5111 mutant with the J-13R cells, whether the replication of the virus is dependent on its interaction with the IL13Rα2 receptor and whether the virus enters by endocytosis or the fusion of the envelope with the plasma membrane. The salient feature of the results and their significance are as follows:

A potentially important finding that may affect future studies designed to target viruses to receptors identified in tumor cells in vivo is that IL13Rα2 was unstable and rapidly lost from transduced cells notwithstanding repeated selections. One factor responsible for the loss of the receptor from the cultured cells appears to be the presence of serum in the medium in which the cells were grown. Following the observation that the IL13Rα2 receptor was lost from the transduced cells, we checked several malignant glioma cell lines and found that none of them exhibited the IL13Rα2 receptor on their surface. A similar decrease was observed in Vero cells transduced and selected for expression of IL13Rα2. The evidence that IL13Rα2 disappeared from the surface of J-13R cells exposed to IL-13 but not following exposure to IL-4 or IL-2 suggests that a ligand in fetal bovine serum binds to the receptor and that the receptor is internalized or degraded. It is noteworthy that another receptor, EGFRvIII, uniquely present in malignant gliomas, is also retained during passage of tumor cells from animal to animal but is lost on cultivation in cultured cells (20). Our results indicate that the cytocidal activity of viruses targeted to IL13Rα2 and potentially to other receptors may have to be measured in tumors in situ rather than in cultured cells.

HSV-1 can enter via three receptors, HveA, nectin1, or a modified form of O-sulfated heparan sulfate (4, 9, 19, 23). J1-1, the parent cell line, lacks these receptors inasmuch as neither the wild-type virus nor R5111 virus infects these cells (24). We have shown here that R5111 recombinant virus retained the capacity to enter cells via the HveA or nectin1 receptors inasmuch as it infected and replicated in J1-1 cells transduced with HveA or nectin1. The experiments described in this report show that R5111 acquired the capacity to infect cells via the IL13Rα2 receptor inasmuch as soluble IL-13 but not IL-4 or IL-2 blocked the multiplication of R5111 recombinant in J-13R cells. In this instance, IL-13 did not compete with the virus but rather caused the disappearance of the receptor from the surface of the J-13R cells.

HSV-1 enters cells by fusion of the virus with the plasma membrane or by endocytosis followed by fusion of the enve-

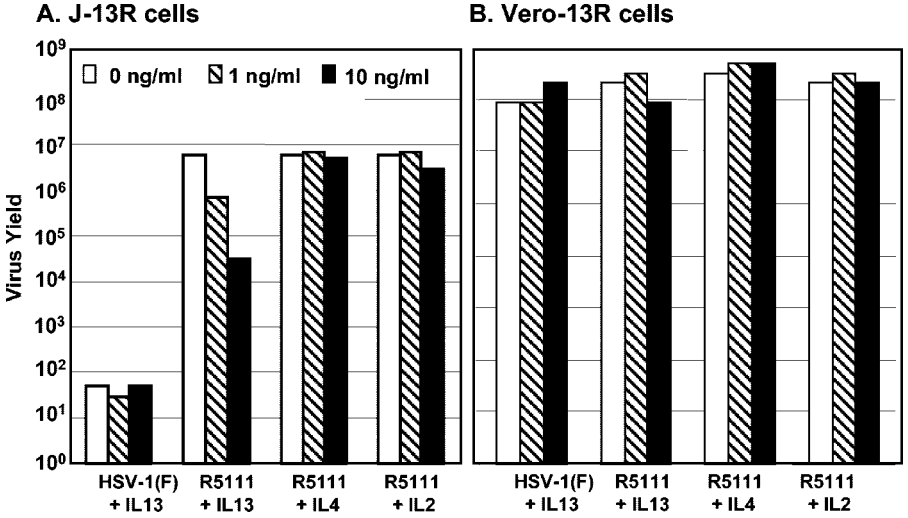

FIG. 7. IL-13 blocks the replication of R5111 in J-13R cells but not in Vero-13R cells. J-13R cells (panel A) and Vero-13R cells (panel B) were either mock treated or exposed for 1 h to either 1 or 10 ng of IL-13, IL-4, or IL-2 per ml. Next, the cells were infected with 0.1 PFU of HSV-1(F) or R5111 recombinant virus per cell and harvested 24 h after infection. Titers of progeny virus were determined on Vero-13R cells.

lope with the membrane of the endocytic vesicle (22). There is a general consensus that fusion with the plasma membrane is the major entry pathway. Entry of virus via the endocytic pathway does not require interaction with a specific receptor, inasmuch as $gD^{-/-}$ virus can be readily visualized in endocytic vesicles within a few minutes after exposure of cells to the virus. Furthermore, in the absence of a complementing gD gene, $gD^{-/-}$ stocks cause the cells to undergo apoptosis. To determine the mechanism of entry of R5111 into cells, we used the PD98059 inhibitor of endocytosis. This inhibitor had no effect on R5111 replication in J-13R cells. In contrast, the inhibitor blocked the replication of $gD^{-/-}$ virus in J1-1 cells transduced with a baculovirus expressing gD.

To our knowledge, this is the first recombinant virus capable of entry into and replication in susceptible cells via a novel receptor. The literature reports other attempts to retarget HSV-1 to novel receptors. In one study, a virus lacking the U_L3-U_L8 open reading frames was pseudotyped with glycoprotein G of vesicular stomatitis virus. This mutant entered gD-complementing cells by endocytosis and at a lower efficiency than the wild-type virus (1). In another study, the same laboratory constructed a virus in which the full-length erythropoietin hormone was fused to an N-terminally truncated gC. The virus was neutralized by antibody to gC of erythropoietin in a complement-dependent fashion and was retained on columns of soluble erythropoietin receptor but failed to infect murine cells exhibiting the receptor (13). In these cells the recombinant virus was endocytosed and degraded. Neither of these viruses meets the criterion of retargeted virus capable of entry into and replication in cells via a novel receptor.

One hypothesis that drives current studies on viral entry is that gD is altered by its interaction with the receptor and that the altered form then interacts with the fusogenic proteins to enable viral entry. One unambiguous conclusion to be drawn from this study is that the change in the structure of gD required to promote the fusion of viral envelope with cellular membrane in conjunction with glycoproteins B, H, and L is not dependent on interactions with specific receptors. Our findings open the way for the construction of recombinant viruses with affinity for other receptors. With respect to the initial objective of these studies, it seems clear that construction of a recombinant totally dependent on IL13Rα2 for its entry should enable the use of more robust viruses retaining an appropriate safety profile and capable of multiplying in dividing as well as nondividing cells.

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