Herpes Simplex Virus Infection Can Occur without Involvement of the Fibroblast Growth Factor Receptor

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Basic fibroblast growth factor (bFGF) has been reported to block uptake of herpes simplex virus type 1 (HSV-1) and plaque formation on arterial smooth muscle cells, suggesting a role for the bFGF receptor in HSV entry (R. J. Kaner, A. Baird, A. Mansukhani, C. Basilico, B. D. Summers, R. Z. Florkiewicz, and D. P. Hajjar, *Science* 248:1410-1413, 1990). We confirmed the effect of bFGF on infection of this cell type with HSV-1 and HSV-2 and found the same result with umbilical vein endothelial cells. However, bFGF does not inhibit plaque formation on any other cell type we tested. Furthermore, there is no correlation between the level of expression of the bFGF receptor and the effect of bFGF. HEp-2 and A431 cells express barely detectable levels of receptor, and yet they are fully permissive for HSV infection in a bFGF-insensitive manner. Thus, interaction of virus with the bFGF receptor is not required for infection of many cell types. In addition, infection of smooth muscle cells is not prevented by incubation of virus with an anti-bFGF antibody, arguing against the hypothesis that virion-associated bFGF acts as a bridge between virus and receptor (A. Baird, R. Z. Florkiewicz, P. A. Maher, R. J. Kaner, and D. P. Hajjar, *Nature [London]* 348:344-346, 1990).

Herpes simplex viruses (HSVs) cause a variety of human diseases, including cold sores, eye and genital infections, and encephalitis; in neonates and immunocompromised patients, infection may be disseminated. The ability of the virus to replicate at numerous sites in the body and its wide host range in cell culture suggest either the use of a common cell surface molecule as a receptor or alternative receptors on different cell types. Since HSV has at least nine glycoproteins, the latter is not implausible; in fact, evidence supporting the existence of two independent receptor pathways on one cell type has recently been published (38).

The following steps have been proposed for virus entry, with varying amounts of evidence. First, virions bind to cell surface heparan sulfate proteoglycans (HSPG), chiefly via glycoprotein C (gC). Next, gD interacts with limiting quantities of a second receptor. Third, the virus envelope fuses with the plasma membrane, with the possible involvement of gB, gD, and gH.

Evidence for the first step is that adsorption of virus to cells is blocked by heparin and is reduced by prior enzymatic removal of heparan sulfate from the cell surface (44). Furthermore, purified HSV and at least two of its glycoproteins (gB and gC) can bind to heparin-Sepharose beads (13).

Several results suggest that gD in virions interacts with a cellular receptor after attachment of the virus to HSPG. First, cell lines expressing large amounts of gD are resistant to infection (1, 4, 17), possibly because intracellular gD sequesters a receptor. Second, infection can be prevented by prior exposure of cells to UV-inactivated wild-type virus but not gD-minus virus (15). Finally, soluble truncated forms of gD-1 and gD-2 show saturable binding to the cell surface; binding of gD, which is unaffected by the presence of heparin, blocks subsequent virus entry but not attachment (14).

The cellular receptor for basic fibroblast growth factor (bFGF) was recently proposed to act as a "portal of entry"

(i.e., receptor) for HSV type 1 (HSV-1) in bovine arterial smooth muscle cells (BSMC) (2, 18). This proposal was attractive for two reasons. First, bFGF receptors are found on many cell types (24, 29, 31, 32, 42), consistent with the ability of HSV to infect most types of cultured cells. Second, bFGF also binds to HSPG (24, 37). Indeed, this appears to be a requirement for subsequent binding to the bFGF receptor (34, 45). Thus, binding of HSV-1 virions might mimic that of bFGF, with virus binding initially to HSPG as a prerequisite for interaction with the receptor. At this point, the pathways would diverge, since binding of bFGF is followed by endocytosis (25, 29), whereas binding of virus is followed by fusion with the plasma membrane (6, 33).

The evidence for an interaction between HSV-1 and the bFGF receptor was twofold. First, bFGF blocked virus uptake and plaque formation on BSMC (18). Second, virus uptake by Chinese hamster ovary (CHO) cells lacking the bFGF receptor was increased after transfection with the receptor gene (18). Furthermore, virus uptake by CHO cells was blocked by a polyclonal antibody against bFGF (2), leading to the proposal that interaction of HSV with the bFGF receptor is mediated by bFGF associated with virions, rather than by a direct interaction between the receptor and a virus glycoprotein.

The results presented here address several points raised by these reports. The ability of bFGF to block plaque formation on BSMC was confirmed and extended; HSV-1 and HSV-2 were both inhibited, and a similar effect was obtained with human endothelial cells. However, inhibition by bFGF was restricted to a minority of cell types. Furthermore, there was no correlation between the amount of bFGF receptor expressed by different cell types and the ability of bFGF to block infection. In fact, some cells permissive for HSV infection had few or no receptors. Therefore, the bFGF receptor is not an absolute requirement for infection. However, bearing in mind the observation by Sears and colleagues (38) that polarized MDCK cells express more than one receptor for HSV, we cannot exclude the possibility that the bFGF receptor plays a role in virus entry in certain cell

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types. Finally, an anti-bFGF monoclonal antibody (MAb) that blocks the binding of bFGF to the receptor and inhibits its biological activity (23) had no effect on virus infectivity, casting doubt on the proposed role of virus-bound bFGF in penetration (2).

MATERIALS AND METHODS

Cells and virus. Vero and MDBK cells were grown in Dulbecco's modified minimal essential medium (DME) containing 5% fetal bovine serum (FBS). A431 cells, a human epidermoid carcinoma cell line (12), and MRC-5 human fibroblasts were grown in DME containing 7.5% FBS. SK-N-MC human neuroblastoma cells and HEP-2 human epidermoid carcinoma cells were grown in DME containing 10% FBS. Bovine smooth muscle cells were grown in minimal essential medium containing 10% FBS. Human umbilical vein endothelial cells were grown on fibronectin-coated plates ($1 \mu\text{g}/\text{cm}^2$) in M199 medium supplemented with 16% FBS and an extract of bovine hypothalamus (46). HSV-1 (KOS and HFEM strains) and HSV-2 (333 strain) were grown and their titers were determined on Vero cells.

Antibodies. DL11 is an anti-gD MAb (28). bFM-1 is an anti-bFGF MAb (23) and was purchased from Upstate Biotechnology Inc., Lake Placid, N.Y. R99 is an anti-bFGF polyclonal serum; it was produced by injecting a rabbit three times with $7.5 \mu\text{g}$ of human recombinant bFGF, the first time in Freund's complete adjuvant and subsequently in Freund's incomplete adjuvant, and then boosting with $1 \mu\text{g}$ of bFGF given intravenously.

Virus growth curves. Cells in 60-mm dishes were inoculated with 2×10^5 PFU of HSV-1 (approximately 0.1 PFU per cell) for 1 h at 37°C , washed twice with complete medium, and then incubated at 37°C for various times. The medium was collected for determination of extracellular virus. To obtain intracellular virus, cells were scraped into cold medium, lysed by one freeze-thaw cycle plus sonication, and then centrifuged at low speed to remove nuclei. Virus titers were subsequently determined on Vero cells.

Inhibition of plaque formation. Cells in 60-mm dishes were washed twice with serum-free medium before the addition of 1 ml of medium containing human recombinant bFGF (Chiron). Monolayers were then incubated with virus for 2 h at 37°C , washed twice with phosphate-buffered saline (PBS), and overlaid with medium containing 5% FBS and 0.6% SeaKem agarose (FMC Corporation). Plaques were counted 36 to 72 h later, depending on the cell type.

Iodination of bFGF. Human recombinant bFGF (Boehringer Mannheim) was iodinated by the Iodogen procedure, essentially as described by Neufeld and Gospodarowicz (29). bFGF ($5 \mu\text{g}$) was reacted with 1 mCi of Na^{125}I and then applied to a 0.5-ml heparin-Sepharose CL-6B column (Pharmacia) equilibrated with 20 mM sodium phosphate (pH 7.2)–0.6 M NaCl. Bound bFGF was subsequently eluted with 20 mM sodium phosphate (pH 7.2)–2 M NaCl. The specific activity was approximately 4.7×10^4 cpm/ng.

Biological activity of ^{125}I -bFGF. Specific activity of the ^{125}I -bFGF was determined by comparison of its biological activity with that of unlabeled bFGF in a cell proliferation assay. Lower sterna chicken embryo chondrocytes were seeded in 35-mm dishes in DME containing 10% FBS. Serial dilutions of unlabeled bFGF (0.15 to $4.5 \text{ ng}/\text{ml}$) or ^{125}I -bFGF (1×10^4 to 3×10^5 cpm/ml) were added 1 h later, and the cells were incubated for 3 days. Hyaluronidase (Calbiochem) was then added to a final concentration of 4 U/ml, and the incubation continued for 6 h. At this time, the cells were

detached by treatment with trypsin-EDTA and counted. The specific activity of the ^{125}I -bFGF was calculated by equivalence of the quantities of labeled and unlabeled bFGF producing half-maximal increases in cell number.

Determination of ^{125}I -bFGF binding. Cells in 24-well plates were washed twice with cold PBS and incubated for 2 h at 4°C with various concentrations of ^{125}I -bFGF in 250 μl of DME–25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.5)–0.15% gelatin (binding buffer). The procedure of Moscatelli (24) was then used to distinguish between binding to HSPG and binding to the bFGF receptor. The cells were washed twice with cold PBS, three times with cold 2 M NaCl–20 mM HEPES (pH 7.5) (to remove ^{125}I -bFGF from HSPG), and twice with cold 2 M NaCl–20 mM sodium acetate (pH 4.0) (to remove ^{125}I -bFGF from the bFGF receptor). ^{125}I -bFGF released by each wash was determined with a gamma counter. Counts per minute due to nonspecifically bound ^{125}I -bFGF (determined on duplicate plates that also received 50 nM unlabeled bFGF) were subtracted from the counts per minute in the combined pH 4.0 washes and normalized to 10^5 cells to allow for differences in cell density. Maximum binding to the receptor was estimated from a semilogarithmic plot of bound versus unbound ^{125}I -bFGF.

Cross-linking. The procedure for cross-linking was essentially that described by Moscatelli and Quarto (26). Cells in 60-mm dishes were incubated for 2 h at 4°C in 2 ml of binding buffer containing 7.5×10^5 cpm of ^{125}I -bFGF. They were then washed with cold PBS and incubated with 0.15 mM disuccinimidyl suberate in PBS for 15 min at room temperature. Cross-linking was terminated by the addition of 200 μl of 10 mM Tris HCl (pH 7.5)–200 mM glycine–2 mM EDTA and then a 1-min incubation at room temperature. After two washes with cold PBS, cell extracts were prepared in 10 mM Tris HCl (pH 7.5)–10 mM NaCl–3 mM MgCl_2 –0.1 M sucrose containing *N*- α -tosyl-L-lysine chloromethyl ketone and *N*-tosyl-L-phenylalanine chloromethyl ketone proteinase inhibitors. Nuclei were removed by low-speed centrifugation, and the supernatants were electrophoresed on a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel.

Virus neutralization. Antibody and virus (approximately 250 PFU) were incubated together for 1 h at room temperature and then added to a monolayer of BSMC. After 1 h at 37°C , the cells were washed with PBS and overlaid with agarose, and plaques were counted 2 days later.

RESULTS

Effect of bFGF on plaque formation on BSMC. BSMC were inoculated with HSV-1 or HSV-2 in the presence or absence of human recombinant bFGF. After 2 h at 37°C , the cells were washed and then overlaid with agarose and incubated at 37°C for 36 h to allow plaque formation. The number of plaques obtained is shown in Table 1. In agreement with the data of Kaner and colleagues (18), we found that bFGF at 100 nM or greater inhibited plaque formation by HSV-1; since we used strains KOS and HFEM, whereas they used strain F, the effect is not strain specific. Neither is it type specific, since HSV-2 strain 333 was also inhibited (Table 1).

Effect of bFGF on plaque formation on other cell types. To determine whether inhibition is restricted to BSMC, we applied the plaque reduction assay to other cell types, using HSV-1 (KOS) as the infecting virus in each case (Table 2). Among the cells used were several of human origin, namely, MRC-5 (fibroblasts), SK-N-MC (neuroblastoma), HEP-2 and A431 (both epidermoid carcinoma), and HUVE (umbilical

TABLE 1. Effect of bFGF on plaque formation in BSMC

bFGF (nM)	Plaque no. ^a		
	HSV-1 (KOS)	HSV-1 (HFEM)	HSV-2 (333)
0	164, 176	214, 290	424, 450
10	160, 150	ND ^b	ND
25	138, 142	ND	ND
100	46, 40	12, 16	50, 42
200	8, 10	8, 8	34, 46

^a Assays were performed in duplicate.^b ND, not done.

vein endothelial). In addition, Vero (African green monkey kidney) and MDBK (bovine kidney) cells were used. For SK-N-MC, HEp-2, and HUVE cells, no agarose was used in the overlay, either because the cells did not grow well under agarose or because the plaques formed under agarose were difficult to visualize. The BSMC control was therefore performed with and without agarose in the overlay. With most cell types, the reduction in plaque number at 200 nM bFGF was 2-fold or less, compared with a 6- to 10-fold reduction with BSMC. A comparable inhibition was seen only with endothelial cells (HUVE), with which the virus titer was reduced 10-fold. Interaction with the bFGF receptor may therefore not be obligatory for entry of HSV into many cell types; an alternative explanation is that an abundance of bFGF receptor molecules on these cells, compared with the number on BSMC or HUVE cells, overwhelms the bFGF used for blocking. To examine this possibility, we performed ¹²⁵I-bFGF binding assays.

Binding of ¹²⁵I-bFGF. As a preliminary experiment, the concentration of unlabeled bFGF needed to block binding of ¹²⁵I-bFGF to its receptor was determined. BSMC in 24-well plates were incubated for 2 h at 4°C with 5×10^5 cpm of ¹²⁵I-bFGF plus various amounts of unlabeled bFGF. The inoculum was then removed, and the cells were washed sequentially with PBS, 2 M NaCl–20 mM HEPES (pH 7.5) (to remove bFGF from HSPG), and 2 M NaCl–20 mM sodium acetate (pH 4.0) (to remove bFGF from its receptor), all at 4°C (24). Each fraction was counted in a gamma counter. The results (Fig. 1) are similar to those found previously for baby hamster kidney cells (24). Unlabeled bFGF at 100 nM reduced binding of ¹²⁵I-bFGF to its receptor

TABLE 2. Cell type specificity of bFGF effect

Cell type	Plaque no. ^a with following concn of bFGF (nM):		
	0	100	200
BSMC (smooth muscle) + agarose	488	ND ^b	50
BSMC (smooth muscle), no agarose	400	110	70
HUVE (endothelial)	210, 188	ND	22, 20
MRC-5 (fibroblast)	210, 228	140, 208	134, 120
A431 (epidermoid)	129, 120	ND	156, 103
HEp-2 (epidermoid)	208, 140	154, 106	132, 124
SK-N-MC (neuroblastoma)	96, 110	138, 98	98, 70
Vero (monkey kidney)	380, 584	336, 440	210, 222
MDBK (bovine kidney)	150, 120	116, 100	132, 132

^a Duplicate dishes were used except with BSMC.^b ND, not done.

by 85%, with a substantial effect even at 1 nM. Binding to HSPG, however, was reduced by less than 50%, even at 100 nM bFGF. Essentially the same result was obtained with Vero cells (data not shown). In a concurrent experiment, the anti-bFGF MAb bFM-1 (23) was present during the binding phase at a concentration of 10 µg/ml (Fig. 1). Its effect was similar to that of 1 nM unlabeled bFGF: binding of ¹²⁵I-bFGF to its receptor fell by 75%, while binding to HSPG was only marginally reduced. As discussed below, this result is significant because bFM-1 had no effect on virus infectivity.

Binding assays were performed with the cell types used in the plaque reduction assays, over a range of 5×10^3 to 2×10^6 cpm of ¹²⁵I-bFGF per well. To account for nonspecific binding, duplicate wells were used, one containing 50 nM unlabeled bFGF to block specific binding to the receptor. After a 2-h binding period, the cells were washed as described above and the fractions were counted. Each cell type displayed binding of ¹²⁵I-bFGF to HSPG, with no sign of saturation even at 2×10^6 cpm per well (data not shown). Specific binding to the bFGF receptor (after subtraction of residual counts in the presence of excess unlabeled bFGF) was found to approach saturation in each case. This is shown by a semilogarithmic Klotz plot (19) of bound versus unbound ¹²⁵I-bFGF (Fig. 2). The specific activity of the ¹²⁵I-bFGF was determined by comparison of its biological activ-

FIG. 1. Inhibition of ¹²⁵I-bFGF binding by unlabeled bFGF and the anti-bFGF MAb bFM-1. BSMC were incubated with ¹²⁵I-bFGF for 2 h at 4°C in the presence of 0 to 200 nM unlabeled bFGF or 10 µg of bFM-1 immunoglobulin G per ml. The cells were then washed sequentially with (i) PBS, (ii) 2 M NaCl–20 mM HEPES (pH 7.5), and (iii) 2 M NaCl–20 mM sodium acetate (pH 4.0). The radioactivity of each fraction was counted in a gamma counter. Fraction iii represents ¹²⁵I-bFGF bound to the bFGF receptor (A), and fraction ii represents ¹²⁵I-bFGF bound to HSPG (B).

FIG. 2. Analysis of ^{125}I -bFGF binding to the bFGF receptor on various cell types. Cells were incubated with various concentrations of ^{125}I -bFGF for 2 h at 4°C and then washed sequentially as described in the legend to Fig. 1. Counts per minute due to nonspecifically bound ^{125}I -bFGF (determined on duplicate plates that received 50 nM unlabeled bFGF in addition to ^{125}I -bFGF) were subtracted from fraction iii, and the counts were normalized to 10^5 cells to allow for differences in cell density. Estimation of the binding plateau for each cell type enabled the calculation of the approximate number of bFGF receptors per cell.

ity with that of unlabeled bFGF (see Materials and Methods), permitting an estimate of the approximate number of receptors per cell. This ranged from about 10,000 for Vero cells down to 300 or less for A431 and HEp-2 cells. Only Vero cells expressed more receptors than BSMC, so the failure of bFGF to block plaque formation on MRC-5, SK-N-MC, MDBK, A431, and HEp-2 cells was not due to an excess of receptors. Binding of ^{125}I -bFGF to endothelial cells could not be studied as they detached from the dish when washed, but cross-linking studies have shown that they do express a bFGF receptor (31).

Cross-linking. Very low levels of receptor were present on A431 and HEp-2 cells. To confirm this finding for A431 cells, we cross-linked ^{125}I -bFGF to the cell surface with the homobifunctional agent disuccinimidyl suberate. A431 and Vero cells were incubated with ^{125}I -bFGF for 2 h at 4°C , plus or minus 25 nM unlabeled bFGF or epidermal growth factor, and then treated with disuccinimidyl suberate. Cytoplasmic extracts were prepared, and the cross-linked species were examined by electrophoresis on a 10% SDS-polyacrylamide gel. The results are shown in Fig. 3. The broad band of 130,000 to 200,000 obtained with Vero cells (lane 1) probably corresponds to the two-domain and three-domain forms of bFGF receptor found in other cell types (8, 16, 22, 26, 29, 32, 35, 36, 42). The faint band observed with A431 cells (lane 4) migrated with the upper part of the band seen with Vero cells, suggesting that they express only the three-domain form. With both cell types, cross-linking was specific as it was inhibited by unlabeled bFGF (lanes 2 and 5) but not by epidermal growth factor (lanes 3 and 6).

Virus growth curves. Despite their relative lack of bFGF receptors, A431 and HEp-2 cells both support HSV replication. The latter are one of the most commonly used cell lines for HSV studies (for example, see references 11, 13, and 21), and A431 cells have been used to study the expression of HSV-induced receptors for Fc and C3b (20). To confirm that HSV-1 replicates efficiently in the A431 cells used here, we obtained virus growth curves for A431 and Vero cells. Cell monolayers were inoculated with a low multiplicity of

FIG. 3. Cross-linking of ^{125}I -bFGF to the bFGF receptor. Vero cells (lanes 1 to 3) and A431 cells (lanes 4 to 6) were incubated with ^{125}I -bFGF for 2 h at 4°C , in the presence or absence of excess unlabeled bFGF or epidermal growth factor, before cross-linking with disuccinimidyl suberate. Cell extracts were then prepared, electrophoresed on a 10% SDS-polyacrylamide gel, and autoradiographed. Lanes 1 and 4, no competing growth factor; lanes 2 and 5, plus unlabeled bFGF; lanes 3 and 6, plus unlabeled epidermal growth factor. K, $\times 10^3$.

HSV-1 (KOS) for 1 h at 37°C , and the inoculum was then replaced with fresh medium. At various times postinfection, the medium containing extracellular virus was removed and the cells were then lysed in cold medium to release intracellular virus. Virus titers were determined on Vero cells (Fig. 4). The rates of intracellular and extracellular virus production and the total yield of virus were very similar in A431 and Vero cells, despite the disparity in bFGF receptor levels.

Virus neutralization. Kaner and colleagues (2) proposed that the interaction between virus and receptor which they described is mediated by virion-associated bFGF, rather than by a virus glycoprotein. This was based on the observation that uptake of HSV-1 by CHO cells expressing the bFGF receptor was inhibited by an antibody raised against an N-terminal peptide of bFGF. However, since CHO cells

FIG. 4. Vero and A431 cells in 60-mm dishes were infected with 2×10^5 PFU of HSV-1 (KOS) for 1 h at 37°C , washed twice, and then incubated at 37°C for various times. The medium was collected for determination of extracellular virus (Δ , Vero; $\square$, A431). To obtain intracellular virus ($\blacktriangle$, Vero; $\blacksquare$, A431), cells were lysed by freeze-thawing and sonication, and nuclei were then removed by centrifugation. Virus titers were subsequently determined on Vero cells.

TABLE 3. Effect of anti-bFGF and anti-gD antibodies on plaque formation

Antibody	Target	Plaque no. ^a
None		248, 220
DL11 (ascites, 10 µl/ml)	gD	0, 0
R99 (serum, 10 µl/ml)	bFGF	206, 296
bFM-1 (purified immunoglobulin G, 20 µg/ml)	bFGF	172, 260

^a Assay performed in duplicate.

also express HSPG, to which the virus can bind (13, 44), it is unclear exactly what the antibody was inhibiting. Furthermore, these cells are nonpermissive for HSV, so the effect of the antibody on infection was not tested. If the proposed role for virion-associated bFGF were correct, then antibodies that prevent bFGF from recognizing its receptor should interfere with virus penetration into permissive cells and thus neutralize viral infectivity. One such MAb, bFM-1, blocks the biological activity of bFGF (23) and in our hands blocks the binding of ¹²⁵I-bFGF to its receptor but not to HSPG (Fig. 1). bFM-1 was therefore tested for virus-neutralizing activity. We also tested R99, an anti-bFGF polyclonal antibody which does not inhibit binding of ¹²⁵I-bFGF to its receptor, and DL11, an anti-HSV gD antibody with potent neutralizing activity (5, 27). Antibody and virus were coincubated for 1 h at room temperature and then added to a monolayer of BSMC. After 1 h at 37°C, the cells were washed with PBS and overlaid with agarose, and plaques were counted 2 days later. The results (Table 3) show that neither of the anti-bFGF antibodies reduced HSV-1 plaque formation, whereas the anti-gD MAb completely blocked infectivity. Thus, virion-associated bFGF is not required for penetration of infectious HSV-1 into BSMC.

DISCUSSION

The cellular receptor for bFGF has been reported to act as a receptor for HSV-1 on BSMC (2, 18). The evidence was as follows. First, bFGF blocked virus uptake and plaque formation, as did a peptide corresponding to residues 103 to 120 of bFGF. Second, virus uptake by cells that normally lack the receptor was increased after transfection with the receptor gene. However, the observations were limited to one cell type and to one strain of HSV-1, so the general importance of a penetration pathway involving the bFGF receptor was unclear. Therefore, the first point addressed in this study was one of virus type specificity. Using BSMC obtained from R. J. Kaner, together with his experimental protocol, we found that bFGF inhibited plaque formation by HSV-1 strains KOS (nonsyncytial) and HFEM (syncytial) and also HSV-2 strain 333 (nonsyncytial). The original observation by Kaner and colleagues (18) was made with HSV-1 strain F, so the effect of bFGF appears not to be strain or type specific. The second point concerned cell specificity. Despite its effect on plaque formation on BSMC, bFGF had little or no effect on HSV-1 plaque formation on the human cell lines HEp-2, A431, SK-N-MC, and MRC-5 or on MDBK (bovine) or Vero (African green monkey) cells. The only other cell type on which plaque formation was inhibited was HUVE cells.

A simple explanation for these results would be that BSMC and HUVE cells express relatively low levels of the bFGF receptor, which can be saturated by 100 to 200 nM

bFGF, whereas the other cell types express substantially more. A binding assay with ¹²⁵I-bFGF showed that this was not the case. The only cell type that expressed more receptor than BSMC was Vero. Expression in SK-N-MC, MRC-5, and MDBK cells was equal to or lower than that in BSMC, and expression in A431 and HEp-2 cells was barely detectable. The result for A431 cells was not surprising, as they have previously been reported to lack a receptor for the endothelial cell growth factor (10), which recognizes the same receptor as bFGF (30). This was the reason for inclusion of A431 cells in the current study. Cross-linking of ¹²⁵I-bFGF to A431 cells confirmed the low level of receptor, and attempts to detect bFGF receptor mRNA in these cells by Northern (RNA) blotting were unsuccessful (7). Despite this, A431 cells were not deficient in virus replication, as shown by comparison of growth curves in A431 and Vero cells. A growth curve was not determined for HEp-2 cells, as they are among the most commonly used cell types for studies of HSV and are clearly not deficient in virus replication.

The failure of bFGF to block plaque formation on many cell types and the finding that two cell lines expressing very low levels of the receptor can be readily infected, in a bFGF-resistant manner, argues strongly that infection can occur without involvement of the bFGF receptor. The crucial cell type during *in vivo* infections is the neuron, since entry of virus into neurons is necessary for the establishment of latent infections within sensory ganglia. We therefore included the human neuroblastoma line SK-N-MC (40) in this study and found that infection of these cells is not sensitive to bFGF. It is possible that bFGF could block infection of neurons *in vivo*, but two recent studies suggest this is unlikely. First, no receptor-mediated retrograde transport of bFGF occurred in rat peripheral sensory, sympathetic, or motor axons (9). Second, although bFGF receptor mRNA was detected in sensory neurons of trigeminal and dorsal root ganglia in rat embryos, no such transcripts were found in any portion of the peripheral nervous system of adult rats (43). It therefore seems doubtful that HSV relies on the bFGF receptor for entry into sensory neurons.

Kaner and colleagues (2, 18) argued that bFGF blocks infection of BSMC by preventing virus interaction with the bFGF receptor, rather than by preventing virus attachment to HSPG, to which bFGF and HSV both bind. However, the fact that 100 to 200 nM bFGF is required to block infection of BSMC, whereas 1 nM bFGF is sufficient to block binding of ¹²⁵I-bFGF to its receptor, seems more consistent with an effect on HSPG. Another piece of evidence for their conclusion was that a peptide corresponding to residues 103 to 120 of bFGF also blocked virus uptake (18). But in a previous study, Baird and colleagues (3) had shown that this peptide binds heparin in a nitrocellulose binding assay; more recently, a 4,000-molecular-weight proteolytic fragment of bFGF that binds heparin-Sepharose was found to contain residues 106 to 120 (41). Inhibition of virus attachment to cells by peptide 103 to 120 may therefore not be a reliable way to discern the mechanism of action of bFGF. Shieh and Spear (39) have also concluded that bFGF probably blocks virus binding to HSPG, based on their finding that expression of the bFGF receptor by a transformed CHO cell line did not result in increased virus binding. Why virus binding to HSPG should be blocked on some cell types and not others is unclear. Perhaps it reflects differences in cell surface levels of HSPG, which are too high to be measured in the ¹²⁵I-bFGF binding assay. Finally, we found that an antibody that inhibits the biological activity of bFGF and

blocks the binding of ^{125}I -bFGF to its receptor did not block HSV infection of BSMC. Therefore, even if infection of a few cell types does involve the bFGF receptor, we think it unlikely that this is mediated by virion-associated bFGF.

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