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Anti-idiotypic antibodies mimicking glycoprotein D of herpes simplex virus identify a cellular protein required for virus spread from cell to cell and virus-induced polykaryocytosis

(penetration/virus receptors)

TIANMIN HUANG AND GABRIELLA CAMPADELLI-FIUME*†

Department of Experimental Pathology, Section of Microbiology and Virology, University of Bologna, Bologna, Italy

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ABSTRACT **Glycoprotein D (gD) of herpes simplex virus 1 (HSV-1) is required for stable attachment and penetration of the virus into susceptible cells after initial binding. We derived anti-idiotypic antibodies to the neutralizing monoclonal antibody HD1 to gD of HSV-1. These antibodies have the properties expected of antibodies against a gD receptor. Specifically, they bind to the surface of HEp-2, Vero, and HeLa cells susceptible to HSV infection and specifically react with a M_r 62,000 protein in these and other (143TK$^-$ and BHK) cell lines. They neutralize virion infectivity, drastically decrease plaque formation by impairing cell-to-cell spread of virions, and reduce polykaryocytosis induced by strain HFEM, which carries a syncytial (*syn*$^-$) mutation. They do not affect HSV growth in a single-step cycle and plaque formation by an unrelated virus, indicating that they specifically affect the interaction of HSV gD with a cell surface receptor. We conclude that the M_r 62,000 cell surface protein interacts with gD to enable spread of HSV-1 from cell to cell and virus-induced polykaryocytosis.**

The cellular receptor involved in attachment and penetration of herpes simplex virus 1 (HSV-1) into susceptible cells has proven to be elusive. First, HSV encodes for at least 10, and possibly 11, glycoproteins and at least 3 nonglycosylated membrane proteins (1, 2), of which only 5 glycosylated proteins appear to be required for viral replication in cells in culture (1, 2). The redundancy of membrane proteins, coupled with results of studies on polarized cells in which some of these proteins are not redundant (3), has led to the hypothesis that susceptible cells have multiple receptors interacting with the same or different virion envelope proteins. Second, approaches based on transfer of cDNAs from susceptible to nonsusceptible cells have not been feasible for dearth of cells lacking receptors for HSV-1 entry.

In an attempt to identify a receptor involved in HSV-1 entry we derived anti-idiotype antibodies to HD1, a potent neutralizing monoclonal antibody to glycoprotein D (gD) (4). The choice was based on the role of gD in virus entry and cell-to-cell spread.

The initial interaction of HSV with susceptible cells involves a low-affinity binding to cell surface heparan sulfate glycosaminoglycans mediated by glycoproteins C (gC) and probably B (gB) (5–7). Likely, this enables a subsequent more stable attachment, which culminates in penetration, possibly by fusion of the virion envelope with plasma membranes (see ref. 8). gD appears to play a significant role in both stable attachment/penetration and spread of virus from cell to cell, as follows. (*i*) Virions devoid of gD can bind cells, very likely to heparan sulfate glycosaminoglycans, but fail to proceed to enter (9). (*ii*) The role in virus spread and in polykaryocytosis induced by syncytium-forming (*syn*$^-$) HSV mutants is supported by several lines of evidence. Thus, monoclonal antibodies to gD reduce plaque formation. Of these, some display neutralizing activity, whereas others do not (4, 10–12). Monoclonal antibodies that reduce plaque formation most frequently also block polykaryocytosis (10, 11, 13). Furthermore, a virus deleted in the gD gene forms plaques only in a cell line expressing competent gD (9). Finally, a recombinant soluble form of gD induces a reduction in the number of plaques if present throughout the interval of plaque development (14). (*iii*) Cells expressing wild-type gD are refractory to infection (15, 16). In these cells viruses attached to cell surface and were taken up into endocytic vesicles, but they were degraded and infection did not ensue (15). The hypothesis which best fit the data was that gD made in these cells sequestered a receptor and prevented the infecting virus from penetrating the cells (15). Subsequent studies of viral mutants selected for their ability to overcome the block to infection imposed by gD and shown to carry a mutation in their own gD gene (17) led to the conclusion that cells may contain more than one molecule capable of interacting with gD (18). The existence of a receptor for gD was also deduced from the observations that UV-inactivated virions carrying gD, but not those devoid of gD, can prevent infection with wild-type virus (19) and that a recombinant anchorless gD bound in a saturable fashion to cells (16). (*v*) Recently, it has been reported that anchorless recombinant gD is phosphorylated and binds mannose phosphate receptors (MPRs) (20) and that antibodies to MPRs and receptors' ligands decreased cell-to-cell spread of infection (21). However, by contrast with recombinant gD, the infected cell gD is only minimally phosphorylated and, surprisingly, cell mutants lacking MPRs were susceptible to HSV infection (21).

We report that a M_r 62,000 protein identified by the anti-idiotypic antibodies plays a role in cell-to-cell spread of virions and polykaryocytosis induced by a mutant *syn*$^-$ HSV-1 strain.

MATERIALS AND METHODS

Production of Anti-Idiotypic Antibodies. New Zealand White female rabbits were injected subcutaneously with 300 μg of HD1 (4) (entire IgGs or Fab fragments, for rabbits 1 and 2, respectively). ELISA titers to HD1 were above 1:80,000 after the third injection. Anti-idiotypic antibodies were purified from sera obtained after the final, eighth, bleeding, according to ref. 22, except that an initial purification of IgGs by affinity chromatography on staphylococcal protein A-agarose gels

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Abbreviations: HSV-1, herpes simplex virus 1; gB, gC, gD, and gE, glycoproteins B, C, D, and E; VSV, vesicular stomatitis virus; CD MPR, cation-dependent mannose phosphate receptor.

*To whom reprint requests should be addressed at: Sezione di Microbiologia e Virologia, Dipartimento di Patologia Sperimentale, Università di Bologna, via San Giacomo 12, 40126 Bologna, Italy.
†Present address: University of Milan, Varese, Italy.

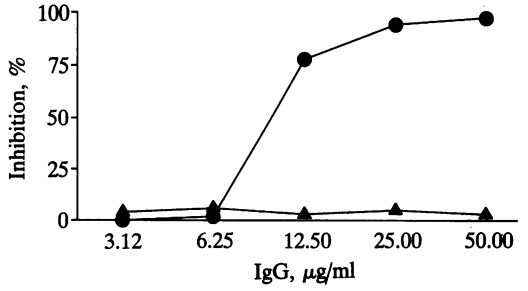

FIG. 1. Binding of anti-idiotypic antibodies to HD1 competes with the binding of HD1 to gD. A constant amount (0.25 μg) of HD1 was incubated with serial dilutions of anti-idiotype (●) or preimmune (▲) IgGs in 0.2 ml of phosphate-buffered saline (PBS) for 1 hr at 37°C, and then allowed to react with affinity-purified gD (62 ng) immobilized onto 96-well trays. Binding was detected with secondary anti-mouse antibody conjugated to peroxidase. gD was purified to homogeneity from HSV-1(F)-infected BHK (baby hamster kidney) cells by immunoaffinity chromatography over monoclonal antibody 30 (18) immobilized on Sepharose, as described (26).

(Pierce) was included. Briefly, purified IgGs were depleted of anti-isotype and anti-allotype antibodies by chromatography over an irrelevant IgG coupled to CNBr-activated Sepharose, purified by immunoaffinity chromatography on HD1 immobilized to CNBr-activated Sepharose, and eluted with 0.1 M glycine, pH 2.8.

Antibodies. Protein A-Sepharose affinity-purified IgG from HD1 and preimmune serum were used throughout this study.

Cells and Viruses. Vero, HEp-2, human osteosarcoma 143TK$^-$, and BHK were grown in Dulbecco's modified Eagle's medium (DME) supplemented with 5% fetal calf serum or 10% newborn calf serum. HSV-1(F) (23) and glycoprotein E-negative (gE$^-$) R7032 (24) have been described. HSV-1(HFEM) was obtained from B. Roizman (University of Chicago).

ELISA. IgG from HD1 (1 μg per well) was immobilized onto the wells overnight in 0.02 M sodium bicarbonate buffer, pH 9.4. Nonspecific binding was blocked with 2% bovine serum albumin. Serial dilutions of immune or preimmune serum were allowed to react with immobilized HD1 at 37°C for 1 hr. Binding was detected by peroxidase-linked anti-rabbit antibody and reading the absorbance at 405 nm. Values representing binding of secondary antibody alone were subtracted. Assays were routinely performed in duplicate or triplicate.

RESULTS

Production of Anti-Idiotype Antibodies. HD1, a potent neutralizing monoclonal antibody to HSV-1 gD, was classified as belonging to the Ia class and reacts with a discontinuous epitope in the N-terminal half of the molecule (residues 1–233) (see ref. 25). Anti-idiotype antibodies elicited in rabbits and purified as described in *Material and Methods* were tested in a competitive ELISA to determine if the fraction of anti-idiotype antibodies reacted with the antigen-binding site of HD1. Serial dilutions of the anti-idiotypic antibody fraction, preincubated with a constant amount of HD1, competed in a dose-dependent manner with the binding of HD1 to immobilized gD, whereas preimmune IgGs did not compete (Fig. 1). We conclude that rabbits produced antibodies to HD1, and that the anti-idiotype-containing fraction (designated as anti-idiotypic antibodies) consisted of antibodies that bear the internal image of gD.

All subsequent experiments were done with antibodies from both rabbit 1 and rabbit 2 and gave essentially similar results, except that antibodies from rabbit 2 gave higher background reactivity with both preimmune and anti-idiotype IgGs in immunoblots (compare Fig. 4*A* with Fig. 4 *B–D*).

Binding of Anti-Idiotype Antibodies to Uninfected Cell Surfaces. Both ELISA and immunofluorescence assays provided evidence that anti-idiotype antibodies specifically react with an antigen on the cell surface. In ELISA, serial dilutions of anti-idiotype antibodies were able to bind to the surface of unfixed or fixed uninfected Vero (African green monkey kidney) or HEp-2 (human epidermoidal) cells in a dose-dependent fashion at concentrations ranging from 85 to 10 μg/ml (Fig. 2 *A* and *B*). At higher concentration binding tended to saturate in Vero cells (Fig. 2*A*). Binding of preimmune IgGs did not exceed 25–30% that of anti-idiotype antibodies (Fig. 2 *A* and *B*). Specificity was assessed in competition experiments where binding of anti-idiotypic an-

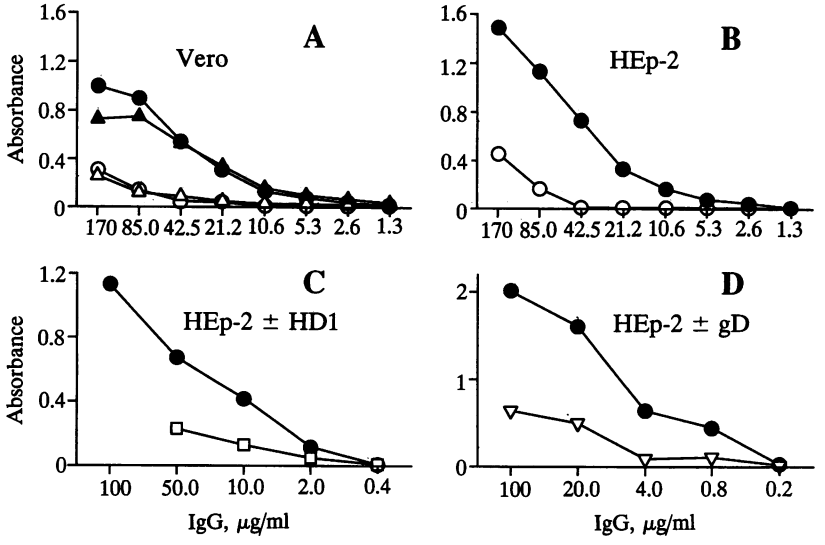

FIG. 2. Binding of anti-idiotype antibodies to uninfected cell surface. Unfixed or 4% formaldehyde-fixed cells, grown on 96-well trays, were allowed to react with serial dilutions of anti-idiotype or preimmune IgGs at 0°C for 1 hr, followed by secondary peroxidase-linked anti-rabbit antibody. Each point represents the average of duplicates. (*A*) Unfixed (●, ○) or fixed (▲, △) Vero cells. (*B*) Unfixed HEp-2 cells. In both *A* and *B*, solid symbols, anti-idiotype antibodies; open symbols, preimmune IgGs. (*C*) Fixed HEp-2 cells. Serial dilutions of anti-idiotype antibodies were preincubated with HD-1 at 100 μg/ml (□) or PBS (●) for 1 hr at 37°C and then allowed to react with cells. (*D*) Fixed HEp-2 cells were allowed to react with purified gD (▽) (4.4 μg in 100 μl of PBS containing 0.1% Triton X-100) or with the same buffer (●) for 1 hr at 0°C, blocked with 1% bovine serum albumin prior to incubation with anti-idiotype antibodies.

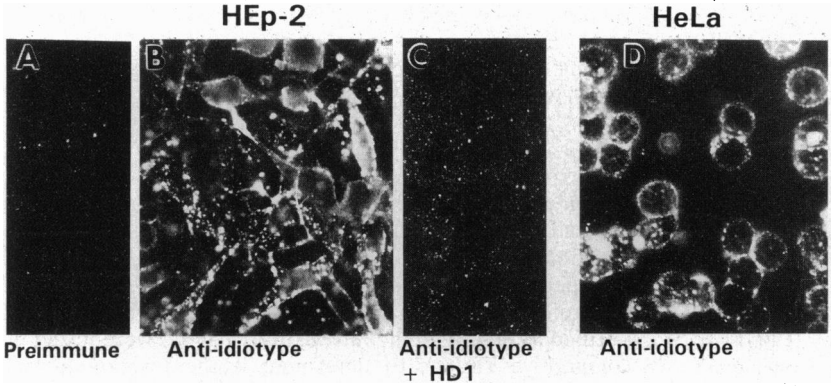

FIG. 3. Indirect immunofluorescence of anti-idiotype antibodies to the surface of HEp-2 (*A–C*) or HeLa (*D*) cells. Cells were grown on glass coverslips, fixed with 2% formaldehyde for 30 min at 4°C, and allowed to react with anti-idiotype (*B–D*) or preimmune (*A*) IgGs (15 μg/40 μl) followed by a fluorescein-conjugated anti-rabbit antibody. In *C*, anti-idiotype antibodies (15 μg) were preincubated with 30 μg of HD1 for 1 hr at 37°C prior to reaction with cells. (×200.)

tibodies to the cell surface was strongly reduced by preincubation of the antibodies with HD1 (Fig. 2*C*) or by preincubation of the cells with gD (Fig. 2*D*).

In immunofluorescence assays, reactivity of anti-idiotypic antibodies to formaldehyde-fixed HEp-2 or HeLa cells was localized to the cell surface with a discontinuous staining (Fig. 3). Binding of preimmune IgGs was negligible. Specificity of the reaction was again assessed by the strong decrease in reactivity following preincubation of anti-idiotypic antibodies with HD1. We conclude that, by these two assays, anti-idiotype antibodies mimicking HSV gD react with a cell surface component of at least three different susceptible cells (HEp-2, Vero, and HeLa cells) and that the reactivity is specific in that it is inhibited by antibodies directed to gD.

Identification of the Specific Protein Reacting with Anti-Idiotype Antibodies. Lysates from HEp-2 cells were separated by denaturing electrophoresis, transferred to nitrocellulose sheets, and allowed to react with anti-idiotype or preimmune IgGs. Fig. 4*A* shows that in addition to two bands reactive with the preimmune IgGs, an intensely stained band with apparent M_r of 62,000 was readily detected by the anti-idiotype antibodies. Specificity was assessed by abolishment of reactivity to the M_r 62,000 protein after preincubation of anti-idiotypic antibodies with HD1 (Fig. 4*B*). Reactivity to the M_r 62,000 protein was detectable in postmicrosomal membrane fraction (Fig. 4*C*).

We next ascertained whether other cells known to be susceptible to infection with HSV-1 express the protein recognized by anti-idiotype antibodies. As shown in Fig. 4*D*, in all cell lines tested (BHK, 143TK$^-$, Vero) the anti-idiotype antibodies constantly reacted with a specific M_r 62,000 protein undetectable by preimmune IgGs. Differences were noted in the reactivity of the preimmune IgGs between Fig. 4*A* (rabbit 1) and *B–D* (rabbit 2), the latter containing much higher background reactivity. Significantly, total cell lysates, and not purified plasma membrane, were employed, except that in *C*, indicating that the protein reacting with anti-idiotype antibodies is present in relatively high abundance. The apparent M_r did not vary in different cell lines, suggesting that the M_r 62,000 protein may be phylogenetically conserved.

Inhibition of HSV Plaque Formation by Anti-Idiotype Antibodies. The evidence that the M_r 62,000 protein recognized by anti-idiotype antibodies plays a role in HSV-1 entry into cells and spread from cell to cell is as follows. (*i*) Anti-idiotype antibodies added from 3 hr prior to virus absorption through the interval of plaque formation caused a significant reduction in the number of plaques, which ranged about from 50% to 100% in different experiments. Preimmune IgGs reduced plaque number by about 25% or less. Results from a typical experiment are shown in Fig. 5*A*. Further experiments were designed to differentiate the effects of antibodies present during virus absorption from those occurring later in infection. Thus, anti-idiotype antibodies added after virus adsorption reduced the number of plaques to a similar extent as antibodies present before and during the entire incubation period (Fig. 5*A*). In assays in which the anti-idiotype antibodies were present solely prior to and during the virus adsorption interval, the inhibitory effect was reduced, but still statistically significantly different from that of controls (Fig. 5*A*). (*ii*) Anti-idiotypic antibodies added immediately (0 hr) or 5 hr after the virus absorption interval yielded comparable high reductions in the number of plaques observed irrespective of the time of exposure of the infected cells to the anti-idiotypic antibodies (Fig. 5 *A* and *B*). It is noteworthy that HD1 abolished plaque formation if added at either 0 or 5 hr after the exposure of virus to cells (Fig. 5*B*), indicating that even the monoclonal antibody to which the anti-idiotypic antibodies were raised has the ability to block cell-to-cell spread of virus, irrespective of its neutralizing effect on virion infectivity.

(*iii*) Since HSV gE can bind the Fc portion of IgGs (27), experiments parallel to those shown in Fig. 5*A* were conducted with the gE-negative mutant R7032 (24). They yielded similar results (not shown), ruling out that binding of anti-idiotype antibodies was nonspecifically mediated by the Fc portion of gE.

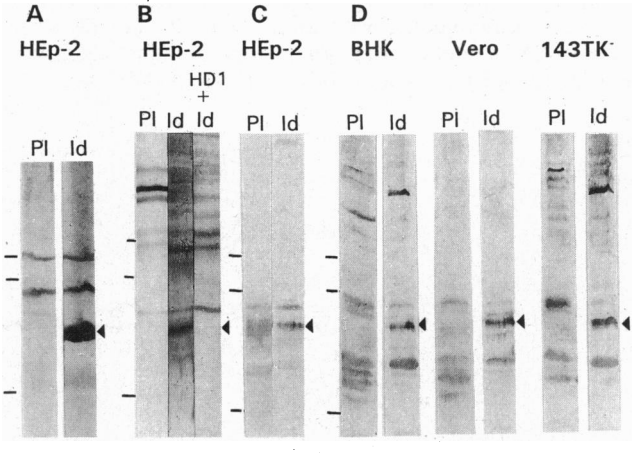

FIG. 4. Identification of the protein reacting with anti-idiotype antibodies. Lysates (*A*, *B*, and *D*) or membranes (*C*) of the indicated cell lines were subjected to denaturing electrophoresis in 8.5% polyacrylamide gels cross-linked with *N,N'*-diallyltartardiamide (26), transferred to nitrocellulose sheets, and allowed to react with anti-idiotype (Id) or preimmune (PI) IgGs, followed by alkaline phosphatase-linked anti-rabbit antibody. (*A*) Rabbit 1. (*B–D*) Rabbit 2. Note the higher background reactivity in lanes with preimmune IgGs of rabbit 2 (*B–D*). In *B*, third lane (HD1 + Id), anti-idiotype antibodies (150 μg/ml) were allowed to react with HD1 (300 μg/ml) for 1 hr at 37°C prior to incubation with nitrocellulose strips. In *C*, membranes from the postmitochondrial (10,000 × *g*) fraction of HEp-2 cells were centrifuged at 100,000 × *g* for 30 min. Bars represent migration positions of rainbow markers of M_r 97,400, 69,000, and 46,000 from Amersham. Triangles point to the specific band identified by anti-idiotype antibodies.

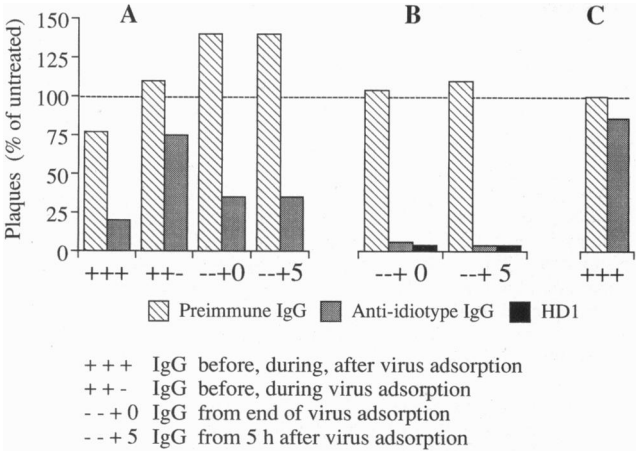

FIG. 5. Effect of anti-idiotype antibodies on development of plaques by HSV-1(F) (*A* and *B*) or vesicular stomatitis virus (VSV) (*C*). Vero cells, grown in 48-well trays, were infected with 50–100 plaque-forming units per well. Anti-idiotype or preimmune IgGs (500 μg/ml) were added to the culture medium 3 hr prior to infection, during virus absorption (60 min), and the 48 hr of plaque development (+++). Alternatively, they were added only prior and during virus absorption (++-), or starting at 0 or 5 hr after the end of virus absorption (--+0, --+5). After removal of viral inoculum, cells were washed with sodium citrate buffer, pH 3, for 1 min, rinsed three times with DME containing 1% heat-inactivated fetal calf serum, and overlaid with the same medium containing anti-idiotypic, preimmune IgGs, or HD1 (35 μg/ml). Pooled human gammaglobulins or methylcellulose was added for HSV plaques or VSV plaque development. Assays were run in quadruplicate. Percentages were calculated relative to untreated controls. Differences were statistically significant, as determined by Student's *t* test ($P < 0.05$), except that in *C*.

(*iv*) Addition of anti-idiotype antibodies to cells infected with an unrelated virus (VSV) had no effect on the number of plaques formed, indicating that the effect of the anti-idiotype antibodies was specific for HSV-1 (Fig. 5*C*). Furthermore, the effects of anti-idiotypic antibodies were independent of the cell line, Vero or HEp-2, in which they were tested (data not shown).

We conclude that anti-idiotype antibodies inhibit plaque formation by two mechanisms. The first, and less potent, blocks the initial infection of a cell with HSV-1. The second, and more potent, mechanism involves blocking the spread of virus from the initially infected cell to adjacent cells by direct contact. This property is shared with HD1, presumably by interaction with different molecules, as HD1 binds gD, whereas anti-idiotypic antibodies mimic gD and bind cell surface components.

Anti-Idiotype Antibodies Reduce HSV-Induced Polykaryocytosis. Several mutations mapping independently in genes specifying glycoproteins have been shown to cause infected cells to fuse into polykaryocytes (see ref. 8). Polykaryocytosis is frequently used as a model of the fusion of viral envelopes with plasma membranes. Some monoclonal antibodies to gD that reduce plaque formation also inhibit polykaryocytosis (10, 11, 13). Of these, some neutralize virion infectivity and some do not (10–12). These observations suggest that the same domain of gD may play a role both in virus spread and in the fusion of infected and uninfected cells. As shown in Fig. 6, the role of gD in HSV-1(HFEM)-induced polykaryocytosis was confirmed and extended by the observation that polykaryocytes were not formed in Vero cells exposed to HD1 at 0 or 5 hr after the end of virus adsorption. Addition of anti-idiotype antibodies strongly reduced the appearance of syncytia (Fig. 6). The inhibition appeared somewhat higher when the anti-idiotype antibodies were added at 0 hr than at 5 hr after the virus adsorption interval. We infer that interaction of gD with the cellular component identified by anti-idiotype antibodies is involved in polykaryocytosis induced by HSV-1(HFEM).

Anti-Idiotype Antibodies Do Not Affect HSV-1 Replication. To rule out the possibility that the reductions in plaque and polykaryocyte was due to a reduction in virus replication, cells infected with HSV-1(F) at 10 plaque-forming units per cell were exposed to the anti-idiotype antibodies from 5 hr after the end of virus adsorption until harvesting at 24 hr. No significant decrease in virus yields at 24 hr was observed under these conditions (data not shown), suggesting that the reductions in plaque and polykaryocyte formation can be attributed specifically to defects in virus transmission and in interaction of plasma membranes of the infected cells with those of the adjacent uninfected cells.

The M_r 62,000 Protein Is Not the Same Protein as the Low Molecular Weight Cation-Dependent Mannose Phosphate Receptor (CD MPR). The CD MPR has a apparent M_r of 46,000. Since variations in apparent M_r are noticed from one laboratory to the other, in this experiment we ruled out the possibility that the M_r 62,000 protein might be the same protein as CD MPR. Lysates from CD-MPR-minus (28) and -plus cells were

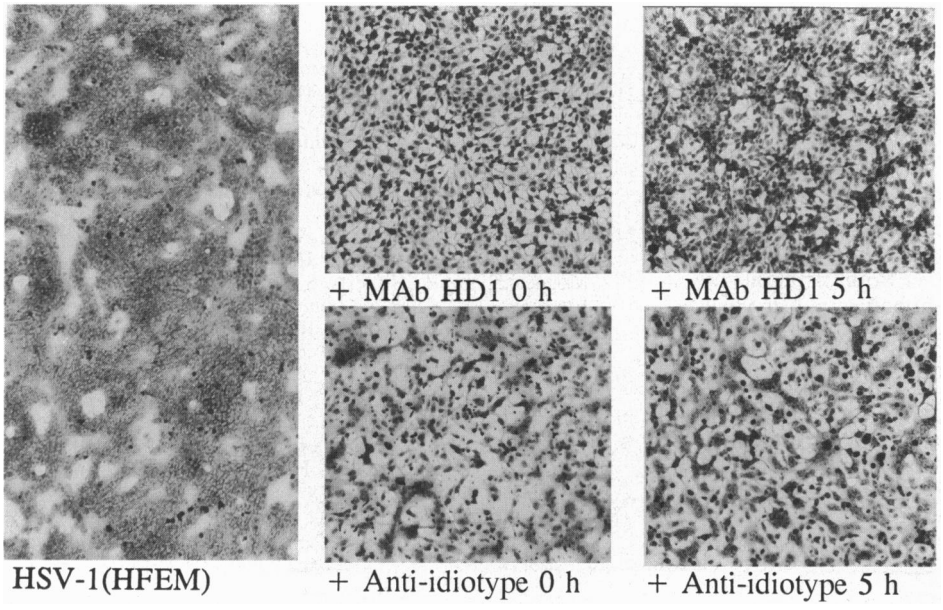

FIG. 6. Effect of anti-idiotype antibodies and HD1 on syncytia formation. Vero cells grown in 24-well trays were infected with HSV-1(HFEM) at 10 plaque-forming units per cell. At 0 or 5 hr after the end of virus absorption, medium was supplemented with anti-idiotypic or preimmune IgG (500 μg/ml) or monoclonal antibody (MAb) HD1 (35 μg/ml). Cells were fixed with methanol 20 hr after infection and stained with Giemsa stain. (All ×60.) The leftmost panel shows a huge syncytium recruiting almost all cells of the culture.

subjected to denaturing electrophoresis and transferred to nitrocellulose. Blotting with anti-idiotype antibodies gave similar patterns in the two samples (not shown), indicating the presence of M_r 62,000 protein in both cell lines.

DISCUSSION

We have presented evidence that HSV interacts with cells through gD by means of a cell surface receptor with apparent M_r of 62,000: (*i*) Anti-idiotypic antibodies mimicking gD bound to the surface of several human, simian, and rodent cell lines susceptible to HSV infection. In each cell line the anti-idiotypic antibodies reacted with a M_r 62,000 protein and binding was inhibited by preincubation of anti-idiotype antibodies with HD1 or of the cells with gD. (*ii*) The anti-idiotypic antibodies neutralized virion infectivity in part, reduced cell-to-cell spread of virus required for plaque formation, and blocked virus-induced polykaryocytosis, as would be expected of antibodies reacting with a specific receptor for gD. Since the antibodies had no effect on a single-step growth cycle of the virus and did not interfere with plaque formation by an unrelated virus (VSV), the anti-idiotypic antibodies affected only the interaction of HSV at attachment/entry of virus into the cells, cell-to-cell spread of virus, and fusion of plasma membranes of cells infected with a *syn*$^-$ virus with those of adjacent uninfected cells. Our studies did not address the question of whether the M_r 62,000 protein is a component of a heteromeric receptor or the sole component of the receptor.

An issue of great interest is the observation that anti-idiotypic antibodies were more effective in inhibiting cell-to-cell spread of virions and polykaryocytosis than initial infection of cells by cell-free virus. It is significant that addition of HD1 as 5 hr after absorption effectively blocked both virus spread and polykaryocytosis, strengthening the view that gD is involved in both phenomena. The localization of gD relative to that of receptor may also differ significantly in uninfected versus infected cells. In the case of attachment of cell-free virus to uninfected cells, the putative receptor, the M_r 62,000 protein, is located in an opposing or "trans" membrane, whereas in the case of cell-to-cell spread of virus the gD may be either on a virus particle in "trans" to the receptor, or on the same membrane, in "cis," and at the same time facing a receptor on the opposing membrane, in "trans." At least three hypotheses may explain our results. (*i*) It is conceivable that the exposure of the M_r 62,000 protein on the uninfected cell surface is minimal. Upon virus attachment, localized changes might occur at the cell surface so that the M_r 62,000 protein becomes available to gD, and hence to the antibodies, whereas the bulk of the receptor remains unavailable to the antibody. By contrast, at the time of cell-to-cell spread of infection, gD is present in large amount on the plasma membrane of the infected cell and may complex with receptors present on the same membranes in "cis" and render them accessible to the antibody. (*ii*) The receptor might be modified during the infection—e.g., by post-translational modifications—or by changes in the relative subcellular distribution, with the result of being more exposed on the plasma membrane late in infection relative to uninfected cells. (*iii*) Both of the above hypotheses require that anti-idiotypic antibodies reduce virus spread and polykaryocytosis by acting on the infected cell. Alternatively, anti-idiotypic antibodies might act on the uninfected cells adjacent to the infected cells. Thus, infected cells might secrete cytokines, or by direct contact, induce adjacent uninfected cells to expose gD receptors to increase the transmission efficiency of the infection. This would render the receptors accessible to the anti-idiotypic antibodies.

While we cannot differentiate between these hypotheses, the identification of the M_r 62,000 protein capable of interacting with gD in both the initial steps of the infection and in cell-to-cell spread of virus opens opportunities to address the mechanisms by which HSV enters cells and is transmitted from infected to adjacent cells.

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1. Roizman, B. & Sears, A. E. (1993) in *The Human Herpesviruses*, eds. Roizman, B., Lopez, C. & Whitley, R. J. (Raven, New York), pp. 11–68.
2. Campadelli-Fiume, G. (1994) in *Encyclopedia of Virology*, eds. Webster, R. G. & Granoff, A. (Academic, London), pp. 603–609.
3. Sears, A. E., McGwire, B. S. & Roizman, B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5087–5091.
4. Pereira, L., Klassen, T. & Baringer, J. R. (1980) *Infect. Immun.* **29,** 724–732.
5. Shieh, M. T., Wudunn, D., Montgomery, R. I., Esko, J. D. & Spear, P. G. (1992) *J. Cell Biol.* **116,** 1273–1281.
6. Herold, B. C., Wudunn, D., Soltys, N. & Spear, P. G. (1991) *J. Virol.* **65,** 1090–1098.
7. Campadelli-Fiume, G., Stirpe, D., Boscaro, A., Avitabile, E., Foà-Tomasi, L., Barker, D. & Roizman, B. (1990) *Virology* **178,** 213–222.
8. Spear, P. G. (1993) in *Viral Fusion Mechanisms*, ed. Bentz, J. (CRC, Boca Raton, FL), pp. 201–232.
9. Ligas, M. & Johnson, D. C. (1988) *J. Virol.* **62,** 1486–1494.
10. Highlander, S. L., Sutherland, S. L., Gage, P. J., Johnson, D. C., Levine, M. & Glorioso, J. C. (1987) *J. Virol.* **61,** 3356–3364.
11. Minson, A. C., Hodgman, T. C., Digard, P., Hancock, D. C., Bell, S. E. & Buckmaster, E. A. (1986) *J. Gen. Virol.* **67,** 1001–1003.
12. Para, M. F., Parish, M. L., Nobel, A. G. & Spear, P. G. (1985) *J. Virol.* **55,** 483–488.
13. Noble, A. G., Lee, G. T.-Y., Sprague, R., Parish, M. L. & Spear, P. G. (1983) *Virology* **129,** 218–224.
14. Johnson, D. C., Burke, R. L. & Gregory, T. (1990) *J. Virol.* **64,** 2569–2576.
15. Campadelli-Fiume, G., Arsenakis, M., Farabegoli, F. & Roizman, B. (1988) *J. Virol.* **62,** 159–167.
16. Johnson, R. M. & Spear, P. G. (1989) *J. Virol.* **63,** 819–827.
17. Campadelli-Fiume, G., Qi, S., Avitabile, E., Foa'-Tomasi, L., Brandimarti, R. & Roizman, B. (1990) *J. Virol.* **64,** 6070–6079.
18. Brandimarti, R., Huang, T., Roizman, B. & Campadelli-Fiume, G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5406–5410.
19. Johnson, D. C. & Ligas, M. W. (1988) *J. Virol.* **62,** 4605–4612.
20. Brunetti, C., Burke, R.-L., Kornfeld, S., Gregory, W., Dingwell, K. S., Masiarz, F. & Johnson, D. C. (1994) *J. Biol. Chem.* **269,** 17067–17074.
21. Brunetti, C. R., Burke, R. L., Hoflack, B., Ludwig, T., Dingwell, K. S. & Johnson, D. C. (1995) *J. Virol.* **69,** 3517–3528.
22. Xue, W., Orten, D. J., Abdelmagid, O. Y., Rider, M., Blecha, F. & Minocha, H. C. (1991) *Vet. Microbiol.* **29,** 201–212.
23. Ejercito, P. M., Kieff, E. D. & Roizman, B. (1968) *J. Gen. Virol.* **2,** 357–364.
24. Meignier, B., Longnecker, R., Mavromara-Nazos, P., Sears, A. & Roizman, B. (1988) *Virology* **162,** 251–254.
25. Muggeridge, M. I., Roberts, S. R., Isola, V. J., Cohen, G. H. & Eisenberg, R. J. (1990) in *Immunochemistry of Viruses*, eds. Van Regenmortel & Neurath, A. R. (Elsevier, Amsterdam), Vol. 2, pp. 459–481.
26. Serafini-Cessi, F., Dall'Olio, F., Malagolini, N., Pereira, P. & Campadelli-Fiume, G. (1988) *J. Gen. Virol.* **69,** 869–877.
27. Baucke, R. B. & Spear, P. G. (1979) *J. Virol.* **32,** 779–789.
28. Ludwig, T., Ovitt, C. E., Bauer, U., Hollinhshed, M., Remmler, J., Lobel, P., Ruther, U. & Hoflack, B. (1993) *EMBO J.* **12,** 5225–5235.