Recovery of an Unusual Fusogenic Herpes Simplex Virus Type 2 Strain from a Clinical Specimen

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A highly unusual herpes simplex virus type 2 strain, strain Burr, was isolated from a female genital tract clinical specimen. This virus induced remarkably rapid and extensive syncytium formation in Vero cells involving hundreds of cells but was less fusion active in HEp-2 cells, MRC-5 cells, and mink lung cells. Virus-infected cells produced the glycoproteins gB, gC, gD and gE.

Membrane fusion is a process used by many viruses for entry into cells, movement of virus between membrane-bound cellular compartments, and egress of virus from cells. Moreover, fusion processes are employed for virus spread in tissues and for recruitment of additional cells to the viral replication cycle.

The known viral fusion proteins have common features; they are composed of one or two type I integral membrane glycoproteins, contain large ectodomains carrying N-linked carbohydrates, form higher-order oligomers, are found in the viral envelope at high surface density, and contain a fusion peptide in a membrane-anchored subunit. Many fusion proteins are made as large precursors which require proteolytic processing (4). Generally, it is thought that a conformational shift exposes a previously cryptic fusion peptide which is then able to insert into the lipid bilayer, initiating the fusion reaction.

Herpesviruses encode numerous different envelope glycoproteins. Eleven glycoproteins have been identified in herpes simplex virus type 1 (HSV-1) and pseudorabies virus. Glycoproteins gC and gD have been found to bind to cell surfaces (3, 9, 11, 13). Virus mutants lacking gB, gD, gH, or gL are unable to penetrate target cells (9, 11). No classical viral fusion protein has been identified for any of the herpesviruses, but reports show that constitutive expression of gB or gD in transgenic cells increases polykaryocyte formation (1, 2).

Glycoprotein gB, a highly conserved protein present in all subfamilies of herpesviruses, is one of the most abundant proteins in the virus envelope and exhibits many of the features described for fusion proteins (10). However, attempts to induce fusion solely with gB have been inconclusive: either gB is not the fusion protein, or fusion requires additional proteins. Other data show that four proteins, gB, gD, and the proteins in the gH-gL complex, have to be present for fusion to occur (12). Moreover, gE/gI localizes virus to the trans-Golgi network and participates in the envelopment of cytosolic nucleocapsids (8), and fusogenic activity is enhanced when a carboxy-terminally truncated version of gB lacking the C-terminal 29 amino acids is used instead of wild-type gB (6).

This paper describes the isolation of a clinical HSV-2 strain, strain Burr, that exhibits unusual fusogenic activity. Because less is known about HSV-2 fusion activity than about HSV-1 fusion activity, this virus may be a good model for the study of HSV-2-induced membrane fusion.

MicroVir Laboratories is a virology reference laboratory to which specimens are submitted by medical providers. The present research complied with all relevant federal and institutional guidelines. Specimens for herpes simplex virus iso-
tion are typically collected on swabs from suspect lesions or from genital mucosa, placed in a liquid viral transport medium, and then submitted for laboratory analysis. The specimen from which HSV-2 strain Burr was isolated was collected from a female genital tract. No information is available on the pathogenesis of the virus in the index patient.

Cultured cell lines were routinely maintained in Dulbecco modified Eagle medium (Sigma Chemical Company, St. Louis, Mo.) supplemented with 2 to 5% Cosmic calf serum (HyClone Laboratories, Logan, Utah). Cells used in this study were Vero cells, HEp-2 cells, mink lung cells (MV1-Lu), and MRC-5 cells. All cell lines were originally obtained from the American Type Culture Collection. Primary isolation of strain Burr was done in Vero cells by using the rapid culture suspension-inoculation technique (5). Stocks of the virus were produced in Vero cells, and all studies reported in this paper were done using a virus stock two passages from primary isolation. Staining of infected cells was done by the immunoperoxidase-based HSV Blu technique (7), by classical indirect immunofluorescence, or by standard histologic or direct tissue stains. The antibody-mediated HSV Blu stain is based on the presence of HSV antigens in infected cells and shows which cells, syncytial or nonsyncytial,

FIG. 2. Vero cells fused by HSV-2 Burr at 48 h postinfection either not stained and visualized by phase-contrast microscopy (A) or stained with immunoperoxidase HSV-Blu (B), Giemsa stain (C), crystal violet (D), Hoechst stain (E), or Sudan black (F). Panels B to F were observed by differential interference contrast microscopy. Nuclear aggregation, cytoplasmic bridging, new cell recruitment, presence of viral antigens, and fused membranes are visible. Original magnification, ×100.
contain detectable antigen, while the histologic stains show the extent of fusion in the syncytia. In addition to HSV-2 strain Burr, other virus strains used were HSV-2 strain 333 (a low-fusion standard laboratory strain), HSV-2 A0260 (a clinical low-fusion isolate), and HSV-1 strain McIntyre. The other three herpesvirus strains were included in the study for comparison of syncytium sizes and presence of viral glycoproteins. The monoclonal antibodies used were anti-HSV(I)gB (Chemicon International, Temecula, Calif.), anti-HSV-2gC2, anti-HSV-2gD, and anti-HSV-2gE-2 (Rumbaugh-Goodwin Institute, Plantation, Fla.), and the HSV culture confirmation and typing reagents were from Wampole Laboratories, Cranbury, N.J. Microscopic images observed by bright-field microscopy, differential interference contrast, and immunofluorescence were captured with a Nikon E600 microscope equipped with a Spot RT slider digital camera. Images observed by phase-contrast microscopy were captured with a Nikon TS100 inverted microscope.

Vero cells infected with strain Burr were stained with either anti-HSV-1 or anti-HSV-2. Micrographs of the results are shown in Fig. 1. The viral focus of infection in Fig. 1B shows that this virus is a type 2 strain (the focus shown in Fig. 1A did not stain with anti-HSV 1), and it also shows syncytium formation with numerous nuclei contained within large cytoplasmic fields. To assess host specificity for fusion activity, strain Burr was grown in Vero cells, HEp-2 cells, MRC-5 cells, and mink lung cells. Fusion activity was subjectively assessed on a scale of 0 to 4+, with 0 indicating no fusion and 4+ indicating syncytia of more than 50 cells at 24 h postinfection. In repeated experiments, the results consistently showed that fusion activity was greatest in Vero cells, least in mink lung cells, and intermediate in HEp-2 cells and MRC-5 cells. It was observed that by 24 h postinfection in Vero cells, large syncytia had formed (Fig. 1B), and by 48 h, vast syncytial formation had occurred; if the cultures were allowed to proceed to completion of cytopathic effect, virtually all of the cells in the cultures were engulfed in very large macrosyncytia. The nature of the appearance of strain Burr syncytia was investigated by examining a variety of unstained and stained foci of infection at 48 h postinfection, shown in Fig. 2. The foci were composed of vast numbers of cells whose membranes were involved in fusogenesis. Typically, near the focal centers were structures composed of aggregated cell nuclei enclosed in a contiguous membrane and thin threads of cytoplasmic material within membranes connecting other nuclear aggregates; around the peripheries of the foci were cells that had individual nuclei but that were still within a sea of common cytoplasm. Cells in the periphery were newly recruited cells recently encompassed in the syncytium; they contained virus-specific antigens and could be thought of as recently captured cells in the enlarging syncytium. Figure 2A shows an infected focus visualized by phase-contrast microscopy, while Fig. 2B shows another focus that has been stained by the antibody-immunoperoxidase technique. The focus in Fig. 2B reveals the cells that contain viral antigens and shows the central aggregate and peripheral recruitment. The focus stained with Giemsa stain (Fig. 2C) shows intensely stained cytoplasm with densely packed nuclei near the center of the focus and more-dispersed nuclei in the focal periphery. Foci with similar appearances are visible in the crystal violet-stained culture shown in Fig. 2D. A focus stained with chromatin-specific Hoechst stain reveals the nuclei of a focus showing nuclear aggregation, but the cytoplasm and the membranes remain unstained. This stain also shows the cultures to be free from mycoplasma contamination. Sudan black attaches to membranes and stains them more intensely than other cellular structures. Figure 2F reveals the mass of individual nuclei in the central aggregate, the presumably contiguous cytoplasmic membrane surrounding the syncytium, and the newly recruited cells in the focal periphery.

Among the major glycoproteins in the herpesvirus envelope that mediate attachment, fusion, and cell-to-cell spread are gB, gC, gD, and gE. Studies to assess the presence of these viral glycoproteins were done with Vero cells, mink lung cells, and HEp-2 cells. If these components were present in strain Burr-infected cells in smaller or extraordinarily larger amounts than those found in cells infected by other herpesviruses, suggestions as to the reasons for the fusogenic activity of strain Burr could be entertained. Four virus strains were tested for these four glycoproteins: HSV-1 McIntyre, HSV-2 333, HSV-2 Burr, and HSV-2 A0260, a slightly fusogenic clinical isolate. Figure 3 shows two foci of infected cells stained by anti-gB that exemplify the appearance of antigen-positive cells. It was found that gB, gC, gD, and gE were all present in detectable amounts in
all cell lines infected with all HSV-2 strains. Cells infected with the HSV-1 strain had low amounts of detectable antigen to anti-gE-2 and anti-gC-2, as expected.

In conclusion, this report describes a highly unusual, fusion-active HSV-2 clinical isolate that may hold promise as an HSV-2 fusion model. Fusion activity was host cell dependent, with Vero cells, of the cells studied, being the most susceptible to fusion. The syncytia formed by this virus were unusually large. Antigens consistent with gB, gC, gD, and gE were present in cells infected with this virus. Molecular studies are under way to further determine the reason for the unusual activity of this virus.

REFERENCES