Role of Adherens Junction Proteins in Differential Herpes Simplex Virus Type 2 Infectivity in Communication-Competent and -Deficient Cell Lines

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Key Words

HSV-2 • Gap junctions • Cx43 • WB cells • HeLa cells • Adherens junctions

Abstract

Background: Gap junctional intercellular communication decreases with HSV-2 infection. To determine the importance of functional gap junctions for infectivity, we compared HSV-2 growth in communication-competent and -deficient cell lines. **Methods:** HSV-2 infectivity was tested in five cell lines: WB rat liver epithelial cells (communication-competent), WB-aB1 (communication-deficient), WB-a/32-10 (communication-rescued), HeLa (communication-deficient), and Cx43-transfected HeLa (communication-rescued) cells. HSV-2 growth curves and indirect immunofluorescence labeling of viral and cell proteins were performed in wild-type and mutant WB cells. **Results:** Although wild-type WB cells were highly permissive for HSV-2 infection, virus production was significantly attenuated in communication-deficient and -rescued mutant WB cells. HeLa exhibited no difference in infectivity between communication-competent and -deficient cell lines. Tight and adherens junction proteins, including zonula occludens-1 and nectin-1, were not different in the WB cell lines. However, E-cadherin levels were elevated

and β -catenin was found to co-localize with glycoprotein E, a viral glycoprotein associated with cell-to-cell spread, in the mutant WB cells. **Conclusions:** These results suggest that attenuated viral production in mutant WB cells is due to viral protein co-localization with adherens junction proteins rather than the loss or restoration of functional gap junctions.

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Introduction

Gap junctions, found in most animal tissues, are clusters of intercellular channels that directly link the cytoplasm of adjacent cells [1]. These channels usually mediate the transfer of low-molecular-mass (~1–2 kDa) ions and metabolites between neighboring cells [2, 3], a process known as gap junctional intercellular communication (GJIC) [1]. More recently, larger molecular weight proteins have been found to permeate gap junctions in vertebrates [4].

Typically, in communication-competent cells such as Cx43-rich, WB-F344 rat liver epithelial (WB) cells, hundreds to thousands of gap junction channels cluster at membrane junctions to form plaques [5]. Following immunofluorescent labeling of Cx43 channel proteins, these

Table 1. Summary of cell lines, functional gap junction communication, HSV-2 infectivity, and membrane protein distribution in this study

Cell type	Gap junction protein in membrane	Functional gap junctions?	HSV-2 infectivity and syncytial formation	ZO-1 in plasma membrane	Nectin-1 in plasma membrane	E-cadherin in plasma membrane	β -Catenin co-localized with gE
WB (wild-type)	Cx43	Yes	High	Yes	Yes	No	No
WB-aB1	Cx43	No	Low	Yes	Yes	Yes	Yes
WB-a/32-10	Cx 43 and Cx32	Yes	Low	Yes	Yes	Yes	Yes
HeLa	None	No	High	ND	ND	ND	ND
Cx43 transfected HeLa	Cx43	Yes	High	ND	ND	ND	ND

ND = Not done.

plaques appear as bright dots or dashes at cell-cell interfaces when viewed with a fluorescence light microscope [6]. In contrast, communication-deficient cell lines such as WB-aB1 (a mutant derivative of WB-F344 [7]), S180 mouse sarcoma [8] and L929 murine fibroblast cells [9] show a more uniform, less punctate distribution of Cx43 at membrane junctions. WB-aB1 cells, like S180 and L929 cells, express normal levels of Cx43 but do not possess the hyperphosphorylated form, Cx43-P2 [7]. The ability of cells to process Cx43 to Cx43-P2 has been strongly correlated to their ability to produce functional gap junctions [10, 11].

A number of effectors can alter the permeability and function of gap junction channels, such as cyclic nucleotides [12] and viral oncogene activity [13]. Following HSV-2 infection, GJIC is significantly attenuated by 6 h post-infection, before morphological changes are evident [14]. Although GJIC may be restored to near control levels when infected cells are treated with an antiviral compound [15], agents that block GJIC do not attenuate infectivity [16]. Stanton et al. [17] demonstrated that Cx43 is significantly downregulated in cytomegalovirus-infected fibroblasts even though these cells do not form gap junctions and Cx43 is localized in intracellular compartments. These conflicting results and the demonstrated reduction in GJIC coincident with viral infection make it necessary to investigate the importance of functional gap junctions in virus infection.

To address this question, we tested HSV-2 infectivity in communication-competent and -deficient cell lines [7, 18, 19]. Communication-competent WB cells were highly permissive to HSV-2 infection while communication-deficient WB cells were less permissive with reduced virus spread. However, both communication-competent and -deficient HeLa cells were highly permissive to HSV-

2 infection. The lack of a direct relationship between functional gap junctions and infectivity led to further exploration of the potential role of tight and adherens junction proteins to explain the difference in infectivity between mutant versus wild-type WB cells.

Materials and Methods

Cells and Cell Culture

Five cell lines were used in this investigation (Table 1): Fischer WB-F344 (WB, GJIC-competent) and WB-aB1 (aB1, GJIC-deficient) cells were a generous gift of Dr. James Trosko, Michigan State University; WB-a/32-10 (a/32-10, GJIC-rescued) cells were kindly provided by Dr. Randall Ruch, Medical College of Ohio, and HeLa cells (GJIC-deficient) and Cx-43-transfected HeLa cells (GJIC-rescued) were generously provided by Dr. Steven Scherer, University of Pennsylvania.

WB cells are rat liver epithelial cells that express Cx43 [18, 20] and carry out very efficient GJIC. WB-aB1 cells are GJIC-deficient mutants that express Cx43 but lack Cx43-P2, the hyperphosphorylated version of Cx43 [7]. WB-a/32-10 cells are WB-aB1 cells that have been stably transfected to express the gap junction protein Cx32 in the presence of Geneticin (Invitrogen Corp., Carlsbad, Calif., USA) [18], which restores GJIC to these cells. WB and aB1 cells were propagated in 75-cm² tissue culture flasks in D-medium (Gibco-BRL) supplemented with penicillin/streptomycin, pyruvate, glucose, NaCl, NaHCO₃ and 10% heat-inactivated newborn calf serum (HyClone), then incubated in a humidified atmosphere with 5% CO₂ in air at 37°. WB-a/32-10 cells were maintained as described above but with the addition of 0.4 mg/ml of Geneticin in D-medium to induce Cx32 expression.

HeLa cells were cultured in minimal essential medium (Gibco-BRL) containing 10% heat-inactivated newborn calf serum (HyClone), 5 U/ml penicillin, and 5 mg/ml streptomycin. Cx43-transfected HeLa cells had puromycin (0.5 μ g/ml) added to the media to induce Cx43 expression. Confluent cells were subcultured regularly. Prior to experimentation, functional gap junctions were determined by using the scrape-loading/dye transfer technique (Table 1) [21].

Fig. 1. Growth curves of HSV-2 in mutant WB cells show reduced virus production at later time points compared to wild-type cells. Early (**a**) and late (**b**) growth curves of HSV-2 (MOI = 0.01) in WB,

aB1, and a/32-10 cells. At various time points, from 2 h to 15 days, cells were frozen at -80° and later assayed for plaque-forming unit ability (PFU) on WB cells.

HSV-2 Growth Curve in Three Cell Types

WB, aB1, and a/32-10 cells were cultured in D-medium and grown to confluence in 25-cm² culture flasks at 37°. The culture medium was decanted from the flasks and 1 ml of HSV-2 strain G (ATCC, 0.01 MOI) was added to each experimental flask while culture medium alone was added to control flasks. After 1 h of incubation at 37°, the virus was removed and replaced with fresh medium. After incubation for 2 h to 15 days at 37°, cells were frozen for later quantification of viral titers which were determined by plaque assay as described below.

Cell extracts from the three HSV-2-infected cell lines frozen at various time points were subjected to three cycles of freezing and thawing, were centrifuged for 10 min at 2,000 rpm, and the supernatant samples were frozen in 1-ml aliquots and stored at -80° . WB cells were then infected with serial dilutions of virus harvested from each cell line and incubated for 36–48 h at 37°, stained with 1% crystal violet in 35% methanol, and analyzed for plaque-forming units.

Indirect Immunofluorescent Labeling

Cells were grown in twelve well plates until near confluence and, when infected, incubated with herpes simplex virus type-2 (HSV-2 Stain G, ATCC, MOI = 10 to maximize infection) for 1 h at 37° in 5% CO₂. Virus was removed and replaced with medium and plates were incubated for 3–48 h at 37°. Cells were washed twice with cold PBS and fixed with methanol for 20 min at -20° . Cells were permeabilized with PBS containing 0.1% (v/v) Tween 20 (PBT) and blocked for at least 30 min with PBT containing 5% fetal calf serum (FCS-PBT). Cells were then incubated for at least 2 h with primary antibody [mouse monoclonal anti-connexin 43, nectin-1, E-cadherin, rabbit ZO-1, β -catenin (Zymed Laboratories, San Francisco, Calif., USA) and rabbit polyclonal anti-HSV-2,

mouse anti-glycoprotein E (Dako Corp., Carpinteria, Calif., USA)] at a dilution of 1:500 in FCS-PBT. Excess antibody was removed by washing plates three times for 5 min each with PBT. Secondary antibodies tagged with AlexaFluor 488 (anti-mouse) and AlexaFluor 546 (anti-rabbit; Molecular Probes, Eugene, Oreg., USA) were added at a dilution of 1:1,000 and incubated at room temperature for at least 1 h or refrigerated overnight.

Western Blot

Protein extracts were separated on a 12% SDS-polyacrylamide gel using a mini-Protein III electrophoresis apparatus (Bio-Rad) run at 100 V. The separated proteins were transferred to PVDF membranes using a mini-Transblot apparatus (Bio-Rad) at 30 V overnight. The membranes were incubated for at least 2 h with a mouse anti-E-cadherin antibody (1:1,000). The bound antibody was detected using an anti-mouse secondary antibody (1:1,000) conjugated with alkaline phosphatase and visualized with the alkaline phosphatase color development reagents, BCIP (5-bromo-4-chloro-3-indoylphosphate *p*-toluidine salt) and NBT (*p*-nitro-blue tetrazolium chloride).

Infectious-Center Assay

Modified infectious-center assays were performed as previously described [22]. Briefly, WB and a/32-10 cells (donor cells) in 35-mm plates at 50% confluence were exposed to virus at 37° to allow entry (MOI = 10). Cells were washed with citrate buffer (pH 3.0) to inactivate residual extracellular virus 1–2 h after infection followed by three washes with PBS and subsequent incubation in medium. Then, 5 h after infection, the infected cells were detached with trypsin-EDTA and counted, and 1,000 cells were plated onto duplicate monolayers of uninfected WB or a/32-10 cells (recipient cells) in medium containing human IgG (Sigma) to

Fig. 2. a–j Cx43 distribution and infection differs among cell types. Indirect immunofluorescent labeling of Cx43 in uninfected WB (**a**), aB1 (**c**), a/32-10 (**e**), HeLa (**g**), Cx43-transfected HeLa (**i**) cells and HSV-2 in infected WB (**b**), aB1 (**d**), a/32-10 (**f**), HeLa (**h**), and Cx43-transfected HeLa (**j**) cells 24 h post-infection. Note the punctate distribution of Cx43 in WB (**a**) and Cx43-transfected HeLa (**i**), the uniform distribution of Cx43 in aB1 (**c**) and a/32-10 (**e**), and the absence of Cx43 in HeLa cells (**g**). WB, HeLa, and Cx43-transfected HeLa were permissive to infection with large viral plaques, whereas aB1 and a/32-10 cells were less permissive with isolated infected cells and absence of cell-to-cell spread (scale bar in **a** = 100 μ m which represents the entire figure).

neutralize infection by virus released into the medium. After 48 h, infected cells were visualized using indirect immunofluorescence with anti-HSV-2 as the primary antibody (see Methods).

Microscopy Techniques

Observations of fluorescent probes were made using an Olympus IX-71 inverted microscope (Olympus America Inc., Melville, N.Y., USA) equipped with epi-illumination fluorescence optics with the proper dichromatic mirrors, excitation filters and barrier filters for visualization of the fluorophores. This microscope was also equipped with an Olympus IX2-DSU spinning disk confocal unit. Images were captured with a Spot Xplorer cooled CCD camera (Diagnostic Instruments Inc., Sterling Heights, Mich., USA), with iVision software (BioVision Technologies Inc., Exton, Pa., USA) running on an Apple Macintosh G-5 computer.

Results

HSV-2 Growth Was Attenuated in Mutant WB Cells at Later Time Points

Both GJIC-deficient aB1 cells and GJIC-rescued a/32-10 cells produced fewer viruses at later time points compared to WB cells (Fig. 1). Low MOI (0.01) was used in this experiment in order to maintain wild-type WB cell viability for the extended time course. Higher MOI (1.0–10) resulted in rapid loss of viable WB cells within 72 h. Although the growth curves for all three cell types were similar for the first 12 h (Fig. 1a), there was at least a 100-fold decrease in virus production in the mutant cells at all of the later time points (Fig. 1b). Based on the similarities of the growth curves at early time points, the mutant cells did not prevent viral entry. The production of fewer viruses later in the infection could be due to differences in cell-to-cell spread or virus yield in infected cells.

Cx43 Expression and HSV-2 Infection in Communicating and Non-Communicating Cells

The presence and distribution of Cx43 in the different cell types are demonstrated in Figure 2. While WB cells exhibited punctate distribution of Cx43 labeling (Fig. 2a), both aB1 and a/32-10 cells showed uniform Cx43 membrane localization (Fig. 2c, e). These results are consistent with previously published studies [6, 7]. HeLa cells showed no membrane Cx43 labeling (Fig. 2g), while Cx43-transfected HeLa cells expressed punctate distribution of Cx43 in the plasma membrane (Fig. 2i). Although WB, HeLa, and Cx-transfected HeLa cells were highly permissive to HSV-2 and produced large virus plaques (Fig. 2b, h, j), aB1 and a/32-10 cells demonstrated few infected cells with markedly reduced virus spread to adjacent cells (Fig. 2d, f). These results (Table 1) indicate that function-

Fig. 3. a–i Distribution differences of Cx43, but not ZO-1, among uninfected cell types. Indirect immunofluorescent labeling of Cx43 (red) in uninfected WB (**a**), aB1 (**d**), a/32-10 (**g**) cells; ZO-1 (green) in uninfected WB (**b**), aB1 (**e**), a/32-10 (**h**) cells, and merged images of Cx43 and ZO-1 (yellow shows co-localization) in uninfected WB (**c**), aB1 (**f**), and a/32-10 (**i**) cells. Note the punctate distribution of Cx43 in WB (**a**), the uniform distribution of Cx43 in aB1 (**d**) and a/32-10 (**g**), and the uniform distribution of ZO-1 in all three cell types. All cell types show evidence of co-localization of Cx43 and ZO-1, although these areas of overlap are fewer in the WB cells due to the punctate distribution of Cx-43 (scale bar in **a** = 25 μ m which represents the entire figure). Colors refer to the online version only.

al gap junctions do not increase HSV-2 infectivity and that another factor is responsible for small plaque formation in the communication-deficient (aB1) and communication-rescued (a/32-10) cells.

Distribution of Tight and Adherens Junction Proteins in WB Cell Lines

The tight junction protein, ZO-1, and adherens junction proteins, nectin-1 and E-cadherin, were evaluated for their cellular localization using indirect immunofluorescence. In contrast to the punctate distribution of Cx43 found only in wild-type cells, ZO-1 was distributed similarly in the plasma membrane of all three WB cell lines (Fig. 3). Nectin-1, an important adherens junction protein and receptor for HSV-2 infection, also was found similarly distributed in both the plasma membrane and nucleus of all WB cells (data not shown). E-cadherin, however, was absent in the plasma membrane of wild-type cells but present in both aB1 and a/32-10 cells (Fig. 4). A Western blot confirming the different expression of E-cadherin in WB versus aB1 and a/32-10 cell lines can be seen in Figure 4g.

Inhibition of Cell-Cell Spread in Mutant WB Cells

Infectious-center assays were performed to test the hypothesis that a/32-10 cells (GJIC-rescued) are resistant to infection by preventing cell-to-cell spread. Cultures of infected WB and a/32-10 cells (donor) were trypsinized and overlaid on recipient WB or a/32-10 cells. When infected WB cells were overlaid on recipient WB cells, large viral plaques, visualized using indirect immunofluorescence, were produced (Fig. 5a). In contrast, infected WB cells (donor) added to a culture of uninfected a/32-10 cells led to small, localized areas of infection (Fig. 5b). When infected a/32-10 cells were overlaid on either uninfected WB cells (Fig. 5c) or a/32-10 (Fig. 5d), small, localized areas of infection are evident. The dual observation that both infected WB cells fail to spread virus to uninfected a/32-10 cells and infected a/32-10 cells do not fuse with and spread virus to uninfected WB cells indicate that the intercellular junctions created by local fusion events between the plasma membrane of infected and neighboring uninfected cells do not occur in a/32-10 cells. These results suggest that mutant WB cells demonstrate reduced viral infectivity at later time points by preventing cell-to-cell spread of virus.

Fig. 4. a–f Mutant WB cells express more membrane E-cadherin compared to wild-type cells. Indirect immunofluorescent labeling of E-cadherin in uninfected WB (**a**), aB1 (**c**), a/32-10 (**e**) cells with merged images of Cx43 (red) and E-cadherin (green) with co-localization shown in yellow in uninfected WB (**b**), aB1 (**d**), and a/32-10 (**f**) cells. Note the lack of plasma membrane-associated E-cadherin in WB (**a**) cells compared to aB1 (**c**) and a/32-10 (**e**) cells. In **g**, Western blot demonstrates less E-cadherin in WB versus aB1 and a/32-10 cells. Compared to WB cells, aB1 and a/32-10 cell types show evidence of co-localization of Cx43 and E-cadherin in the plasma membrane (scale bar in **a** = 100 μ m which represents the entire figure). Colors refer to the online version only.

Co-Localization of β -Catenin with HSV-2 Glycoprotein E

Due to the plasma membrane localization of E-cadherin and decreased cell-to-cell spread of HSV-2 in mutant WB cells, β -catenin, a protein associated with E-cadherin, and glycoprotein E (gE), a protein important for cell-to-cell spread of HSV-2 [23–26], were investigated for their ability to interact with each other in infected cells. As seen in Figure 6a and b, membrane fusion and syncytial formation in infected wild-type WB

cells was associated with loss of membrane β -catenin and no co-localization of β -catenin with gE. In contrast, mutant WB cells demonstrated co-localization of β -catenin and gE in infected cells 48 h post-infection (Fig. 6d, f).

Table 1 provides a summary of the results highlighting the differences between the cell types with respect to their gap junction proteins, communication, infectivity, and membrane protein distribution.

Fig. 5. a–d Cell-to-cell spread of HSV-2 is reduced in mutant cells. Infectious-center assay with infected cells labeled via indirect immunofluorescence using anti-HSV-2. Infected WB cells served as the donor cells in **a** and **b** while infected a/32-10 cells were the donor cells in **c** and **d**. In **a**, infected WB donor cells were seeded onto uninfected, recipient WB cells, while in **b**, infected WB cells were seeded onto uninfected, recipient a/32-10 cells. In **c**, infected a/32-10 cells were seeded onto uninfected WB cells, while in **d**, infected a/32-10 cells were seeded onto uninfected a/32-10 cells. Note the large infected area in **a**, but small, punctuate infected areas in **b**, **c**, and **d** (scale bar in **a** = 100 μ m which represents the entire figure).

Discussion

Loss of functional gap junctions following infection may serve as a cellular protective mechanism to limit viral spread. Previous studies have demonstrated that, by 3 h post-infection and before morphological changes take place, GJIC was attenuated in HSV-2 infected Vero cells [14]. In addition, Cx43 was lost from the plasma membrane at 24 h post-infection [16].

In an attempt to elucidate the relationship, if any, between functional gap junctions and HSV-2 infectivity, we compared HSV-2 growth in WB cells and in a GJIC-deficient mutant, WB-aB1. WB-aB1 cells were markedly more resistant to HSV-2 infection compared to wild-type WB cells. In WB-a/32-10 cells, restoring GJIC by transfection with the alternate gap-junction protein Cx32 did not reverse the resistant phenotype of aB1 cells (Table 1). Although the mechanism responsible for the loss of communication in aB1 cells is not known [J. Trosko, pers. commun.], we also found that communication-deficient HeLa cells and HeLa transfected with Cx43 were equally permissive to HSV-2. The combination of these results and the lack of a direct relationship between Cx43 or GJIC and infectivity led us to investigate other gap junction-associated proteins to explain the different infectivity in WB cells.

The formation of functional gap junctions depends on the presence of tight and adherens junctions, making intercellular junctional proteins an important component of cellular communication [27]. Tight junction proteins, such as zonula occludens-1 (ZO-1), tether transmembrane proteins to the actin cytoskeleton and a growing number of connexins can associate with ZO proteins to organize gap junctions and/or regulate intercellular communication [26, 28]. Despite the important role in communication played by ZO-1, no difference in ZO-1 distribution was found in wild-type versus mutant WB cells in the present study.

Adherens junctions mediate adhesion between cells by linking the actin cytoskeleton of one cell to that of the next cell via transmembrane adhesion proteins and their associated protein complexes, such as the nectin/afadin and cadherin/catenin complexes [27]. A recent review by Gonzalez-Mariscal et al. [29] highlights the importance of adherens junctions in viral entry and spread. Nectins serve not only as cellular entry receptors for HSV [30], but also as mediators of the viral cell-to-cell spread [24]. In this study, no difference in nectin-1 distribution was found between wild-type and mutant WB cells, suggesting that another membrane-associated protein was responsible for differences in infectivity.

The importance of nectin-1 for HSV infectivity and its interaction with cadherin led us to investigate variations

Fig. 6. a–f β -Catenin and gE co-localize in mutant cells. Indirect immunofluorescent labeling of β -catenin (red) in infected WB (**a**), aB1 (**c**), or a/32-10 (**e**) cells with merged images of β -catenin and gE (green) in infected WB (**b**), aB1 (**d**), and a/32-10 (**f**) cells 48 h post-infection. Note the loss of β -catenin in the plasma membrane and lack of co-localization of gE and β -catenin in infected WB cells compared to co-localization of gE and β -catenin (yellow) in infected aB1 (**c**) and a/32-10 (**e**) cells (scale bar in **a** = 100 μ m which represents the entire figure). Colors refer to the online version only.

in cadherin levels and distribution in WB cells. Both aB1 and a/32-10 cells demonstrated greater protein content and plasma membrane distribution of cadherin compared to wild-type cells. Cadherins comprise an important family of transmembrane glycoproteins that mediate calcium-dependent cell-cell adhesion, are linked to the actin cytoskeleton via catenins, and serve as docking sites for gap junctions [31]. Meyer et al. [32] observed in Novikoff cells that antibodies directed against either the extracellular domain of Cx43 or N-cadherin prevented both gap junction and adherens junction formation. In aB1 and a/32-10 cells, the uniform distribution of E-cadherin in the plasma membranes may be responsible for the even pattern of Cx43 distribution in uninfected cells.

Our results from both the growth curves and the infectious-center assay suggest that the decreased HSV infectivity in mutant WB cells is due to attenuated viral

spread. The viral glycoproteins, gE and gI, mediate viral spread through interactions with junctional proteins [23–25, 33]. Since cadherin was absent from the plasma membrane in wild-type WB cells, we investigated the ability of β -catenin, another AJ protein that associates with cadherin [34], to mediate virus spread in infected WB cells. After 48 h post-infection, β -catenin and gE remained co-localized in infected aB1 and a/32-10 cells but not in wild-type cells. Co-localization of gE and β -catenin at membrane junctions was found in previous studies [24] but only after 11 h of infection with wild-type HSV-1 [35]. In addition, Wisner et al. [36] showed that plaque formation and cell-to-cell spread by an HSV gE[−] mutant depended on whether the cells possessed extensive junctions. In a later study, Farnsworth and Johnson [25] found different plaque sizes, β -catenin distribution and trafficking of gE/gI to cell junctions depending on whether they used hu-

man keratinocytes (HaCaT) or retinal epithelial (ARPE-19) cells. They observed greater co-localization of mutant gE with cytoplasmic β -catenin in ARPE-19 cells which was associated with smaller plaque size [25]. In the present study, decreased cell-to-cell spread of wild-type HSV-2 in mutant WB cells was associated with the presence of plasma membrane cadherin and co-localization of gE and β -catenin 48 h following infection. These results suggest that, compared to wild-type cells, mutant WB cells possess a mutation that permits tighter association between gE and β -catenin which prevents cell-to-cell spread.

In summary, HSV-2 infection causes loss of functional gap junctions and plasma membrane Cx43 in wild-type WB cells early in infection, but the ability of virus to infect cells is not directly dependent on the presence of functional gap junctions or Cx43. Mutant WB cells show altered infectivity characterized by deficient cell-to-cell

spread that is associated with increased membrane cadherin and co-localization of gE and β -catenin following long periods of infection. These cells could serve as useful tools for identifying cellular genes that are required for virus fusion events and the interaction of viral gene products with membrane proteins. In addition, treatments that target adherens junction interactions with viral proteins should be investigated as a novel mechanism to attenuate HSV-2 cell-to-cell spread.

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