Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 62 (2008) 167–176
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Reconsideration of viral protein immunoblotting for differentiation of human herpes simplex viruses

Robert N. Bowles, Jamie C. Yedowitz, John A. Blaho*,[1]

Department of Microbiology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574, USA

Received 7 April 2008; accepted 4 June 2008

Abstract

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are ubiquitous human pathogens that infect their hosts for life and reactivate to cause disease at or near the initial site of infection. As the incidence of genital HSV-1 infections increase, there is an increased demand for valid viral typing diagnostics. In this report, we reconsidered and developed a triple-phase immune-typing procedure that compares differences in electrophoretic mobilities of viral ICP4, ICP27, and VP22 proteins between HSV-1 and HSV-2 strains. We isolated and immunotyped 5 primary HSV-1 strains derived from orofacial, ocular, and genital areas along with 2 primary HSV-2 strains from the genital area. Advantages of this methodology include its general technical simplicity, sensitivity, and ability to definitively type HSV. It is anticipated that this methodology will be useful in distinguishing viruses obtained in clinical cultures.

Keywords: HSV-1; HSV-2; Diagnostics; Viral protein; Immunoblotting

1. Introduction

Herpes simplex viruses (HSVs) cause widespread infections in the human population. In the case of HSV type 1 (HSV-1), the severity can range from simple cold sores to fatal encephalitis (Markoulatos et al., 1997). Vertical transmission of HSV type 2 (HSV-2) can result in disseminated neonatal infections, which can have devastating consequences (Corey, 1994; Coyle et al., 1999). In each situation, the virus is spread by intimate oral/sexual contact, requiring penetration of the virus through skin or breaks in the mucous membrane. Once infection has initiated, the virus may migrate through innervating neuronal axons to the host ganglia where it lies dormant until reactivation (Dawkins, 1990). During a productive lytic infection in cultured cells, HSV replication follows a highly ordered program that involves tight transcriptional regulation, which proceeds in a cascade fashion (Roizman, 2001). HSV gene expression begins with the immediate-early (IE) genes whose transcription occurs in the absence of de novo protein synthesis (Batterson and Roizman, 1983; Honess and Roizman, 1974, 1975). The IE gene products, which include infected cell protein (ICP) 4 and 27, cooperatively act to regulate all kinetic classes of viral gene expression (Roizman, 2001). Synthesized next are the early (E) gene products, which encode proteins that are mainly associated with viral DNA synthesis (Boehmer and Lehman, 1997). The late (L) gene products, such as the major tegument virion protein (VP) 22, are the last set of viral proteins produced and are principally involved in virion assembly and structure (Enquist et al., 1998). Completion of the viral replication cycle ultimately results in the destruction of infected cells.

Advances in the field of molecular pathology are leading to developments in a number of polymerase chain reaction (PCR)-based differentiation techniques (Espy et al., 2000; Kessler et al., 2000; Kimberlin et al., 1996; Lakeman and Whitley, 1995; van Doornum et al., 2003). PCR-based restriction fragment length polymorphism, in particular, is a sensitive method for typing HSV in the clinic (Madhavan et al., 2003; Marshall et al., 2001).

* Corresponding author. Tel.: +1-212-241-7319; fax: +1-212-534-1684.
E-mail addresses: john.blaho@mssm.edu, jblaho@mdlab.com (J.A. Blaho).
[1] Present address: Virology Research Division, MDL Corporation, 2439 Kuser Road, Hamilton, NJ 08690-3303.

0732-8893/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.diagmicrobio.2008.06.005

Although these assays require instrumentation, experienced technical support, and isolated work areas to prevent contamination, the detection of HSV DNA in cerebral spinal fluid is considered the "gold standard" for diagnosing HSV encephalitis in the clinical virology laboratory (reviewed in Tyler, 2004; Whitley, 2006).

Detection of HSV from the skin and mucosa by PCR methods has been used routinely for over a decade (Hobson et al., 1997). More recently, quantitative real-time PCR tests have significantly improved the sensitivity and selectively of these assays (Adelson et al., 2005; Espy et al., 2000; Filen et al., 2004; Jerome et al., 2002; Lai et al., 2005). Although this argues strongly for HSV PCR to be the standard for all HSV diagnostics, virus isolation from mucosa and skin in shell cultures is still used in many clinical settings. Unfortunately, virus growth in cultured cells cannot distinguish between HSV-1 and HSV-2, so distinctions are often made based on clinical presentation.

In this study, we reconsidered the use of viral protein immunoblotting for differentiating HSVs. The original HSV-specific monoclonal antibodies were directed against HSV-1 antigens. It was almost immediately recognized that these new reagents cross-reacted with polypeptides from HSV-2, and it was proposed that they might serve as a means to differentiate HSV types, if used for Western blotting (Pereira et al., 1976, 1977). In our hands, immunoblot typing has been extremely useful for gene identification when combined with a panel of HSV-1 × HSV-2 intertypic recombinant strains (Blaho et al., 1994). We now present the definitive immunotyping 5 HSV-1 and 2 HSV-2 clinical isolates using the viral protein immunoblot technique. This method should complement and, perhaps, assist in validating current DNA-based assays for clinical virus typing.

2. Materials and methods

2.1. Cell culture

Vero cells were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin. Unless otherwise noted, all cell culture reagents were purchased from Life Technologies, Bethesda, MD. HSV-1(F) and HSV-2(G) are standard laboratory strains (Ejercito et al., 1968). HSV-2(333) is another laboratory-passaged virus, which has been routinely used as a standard strain (Duff and Rapp, 1971; Oram et al., 2000). To obtain high titer virus stocks, we inoculated confluent Vero monolayer cells (approximately 2×10^7) with a multiplicity of infection (MOI) of 0.01 for 2 h at 37 °C in a medium containing 5% newborn calf serum (NBCS). The inoculum was then removed, fresh 5% NBCS was added, and the cells were incubated at 37 °C in 5% CO_2. Virus stocks were prepared once the infection reached a cytopathic effect of 100%. The infected cells were lysed by freezing at −80 °C. After allowing them to warm to room temperature and transferring to a tube containing 3 mL of sterile 9% milk (Carnation nonfat, dry; Glendale, CA), extracts were sonicated (3 × 10 s with cooling periods) using a Branson Sonifier 250 (output level 4) on ice to release the virus particles. Virus stocks were aliquoted and stored at −80 °C. The virus titers were established from the number of plaque-forming units (PFU) detected with Vero cells after serial dilutions. An Olympus (Melville, NY) CK2 inverted phase-contrast microscope was used to count and pick viral plaques.

2.2. Isolation of HSV

HSV was isolated from clinical lesions as follows. After disinfecting the scab and surrounding area with ethanol, the scab was then removed by scraping with the end of a sterile swab. The lesion was then swabbed and the swab used to immediately inoculate 3 mL of 5% NBCS, which in turn was used to inoculate a confluent layer of Vero cells in a 25-cm^2 flask (approximately 4×10^6 cells). The virus and cells were incubated with rocking at 37 °C, after which time, the medium was aspirated and replaced with 3 mL of fresh 5% NBCS, followed by incubation at 37 °C in 5% CO_2 until the infection reached a cytopathic effect of approximately 100% (4 to 9 days). An original virus stock was prepared and its titer determined as described above. The isolation of all viruses in this study was performed in accordance with the guidelines of our institutional review board.

2.3. Plaque purifications

Serial dilutions of the HSV stocks were made in 5% NBCS (3 mL) and used to inoculate a series of 25 cm^2 flasks containing confluent Vero cells. The cells were incubated with rocking at 37 °C for 2 h, the medium was replaced with fresh-containing 2.5 μg/mL pooled human immunoglobulin (Sigma, St. Louis, MO), followed by incubation at 37 °C in 5% CO_2 for approximately 2 days. After this time, an appropriate dish containing well-separated plaques was selected, the medium aspirated, and the cells overlaid with 1% agarose in sterile phosphate-buffered saline (PBS). A number of plaques were picked and used to inoculate separate tubes of 5% NBCS/sterile 9% milk (1:1, 2 mL), which were subject to sonication for 30 s (on ice) using a Branson Sonifier 250 (output level 4). From each of these viral suspensions, 0.5 mL was taken and used to infect individual 75-cm^2 flasks of confluent Vero cells, and high titer virus stocks were prepared as above. One of these stocks was selected and designated plaque-purified passage A.

2.4. Whole cell extracts

Confluent 25 cm^2 flasks of Vero cells were infected with viruses at an MOI of 5 in 5% NBCS. For mock infections, the same procedure was followed, but without addition of virus to the NBCS. After a 2-h adsorption on a