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ORIGINAL ARTICLE

Expression of variable viruses as herpes simplex glycoprotein D and varicella zoster gE glycoprotein using a novel plasmid based expression system in insect cell

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Abstract Several prokaryotic and eukaryotic expression systems have been used for in vitro production of viruses' proteins. However eukaryotic expression system was always the first choice for production of proteins that undergo post-translational modification such as glycosylation. Recombinant baculoviruses have been widely used as safe vectors to express heterologous genes in the culture of insect cells, but the manipulation involved in creating, titrating, and amplifying viral stocks make it time consuming and laborious. Therefore, to facilitate rapid expression in insect cell, a plasmid based expression system was used to express herpes simplex type 1 glycoprotein D (HSV-1 gD) and varicella zoster glycoprotein E (VZV gE). Recombinant plasmids were generated, transfected into insect cells (SF9), and both glycoproteins were expressed 48 h post-infection. A protein with approximately molecular weight of 64-kDa and 98-kDa for HSV-1 gD and VZV gE respectively was expressed and confirmed by SDS. Proteins were detected in insect cells cytoplasm and outer membrane by immunofluorescence. The antigenicity and immunoreactivity of each protein were

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confirmed by immunoblot and ELISA. Results suggest that this system can be an alternative to the traditional baculovirus expression for small scale expression system in insect cells.

1. Introduction

Herpes simplex viruses (HSV) and varicella-zoster virus (VZV) are responsible for a wide range of human diseases with overlapping degree in clinical presentations and the human pathogen that can produce a wide range of mucocutaneous manifestations. The infections extending from skin and genital lesions to the central nervous system, can be caused by bacteria, viruses, fungi, or parasites (Wong et al., 2016). The biological and scientific nature of the disorders caused by these viruses has been elucidated, at least in part, during the past 150 years. The researchers are proving the infectious nature of the pathogens, understanding the basic concepts of latency and reactivation, devising specific antiviral agents, and sequencing the entire viral genomes were all products of the biological and molecular revolution of biomedical research of the 20th century. Although herpes infections do not attract the level of public attention that they did before the era of HIV/AIDS, they are still of major medical importance in the realms of dermatology, paediatrics, infectious diseases, obstetrics and gynaecology, and neurology (Steiner et al., 2007). Herpes simplex type 1 is responsible for 90–95% of cases while herpes simplex type 2 (HSV-2) contributes the remaining 5–10% of herpes simplex encephalitis (HSE) cases (Stone and Hawkins, 2007). Meningitis, encephalitis, and myelitis are caused by HSV viruses in the CNS disease. HSV-2 has more often been linked to recurrent aseptic meningitis than HSV-1 and causes neurological complications more often than most other viruses. However, HSV-2 meningitis has also been recognised as a significant cause of morbidity and mortality in immunocompromised patients (Akya et al., 2015).

VZV a member of herpesvirus family causes chickenpox (varicella), generally in infants, may reactivate decades later to produce shingles (zoster). The neurological complications of VZV are rarely seen during the primary infection (varicella cerebellitis) and more often during the reactivation phase. However, most severe neurological complications occur in immunocompromised patients and include aseptic meningitis, encephalitis with vasculitis, ventriculitis, severe necrotising myelitis, post-herpetic neuralgia and leukoencephalopathy (Corti et al., 2015).

Acute disseminated encephalomyelitis (ADEM) is an uncommon disorder of the central nervous system that occurs after viral illness or vaccination. ADEM resulting from VZV vaccination is estimated to occur in 1 in every 1000 cases of ADEM (Idrissova et al., 2003). Symptoms usually occur after days (2–30 days) of neurological illness and are characterised by an acute onset of focal neurological signs and encephalopathy (Sonneville et al., 2009). Symptoms include; neck stiffness, seizures and fever, and although the latter is not a constant feature, it is present in 75% of severe cases (Sonneville et al., 2009). Clinical diagnosis of these infections is mostly based on the presence of characteristic vascular eruption; however, it can be similar to other viral or nonviral skin infections (Crum-Cianflone, 2008) Therefore, confirmatory test is needed, this can be done by a number of laboratory tests such as virus isolation, direct or indirect immunofluorescence, detecting viral DNA by polymerase chain reaction (PCR), and detecting antibody responses to the viruses using enzyme-linked immunosorbant assay (ELISA). Serological assays using viral antigen play a role in diagnosis and epidemiological studies, however these assays are varying in sensitivity, specificity and in the methodology for detecting HSV and VZV antibody.

Over the past 20 years, the baculovirus expression system has become one of the most popular vehicles for the production of large quantities of recombinant protein. Baculovirus protein expression is a eukaryotic based expression system and thus offers protein modification and a processing pattern similar to those of higher eukaryotic cells. Although recent advances in baculovirus technology include the development of a wide variety of transfer vectors and cloning methods, simplified recombinant virus isolation, quantification methods, and development of cell culture technology have improved. However, the manipulation involved in creating, titrating, and amplifying viral stocks makes it time consuming and laborious. Therefore, in this study a plasmid based expression system InsectDirect (Novagen, Merck, USA) was used to express the full length of HSV-1 gD and VZV gE recombinant glycoproteins in insect cells. The present study demonstrates that the InsectDirect system employs an optimal expression vector with high efficiency for transfection and it seems to be an ideal system for rapid expression of virus glycoproteins in insect cells.

2. Materials and methods

2.1. Genotyping of HSV-1 gD and VZV gE DNA fragment

Genotyping was performed with specific primers used to amplify the full open reading frame of each DNA region encodes HSV-1 gD and VZV gE proteins, for HSV-1 gD a sense primer HSVgDF (5′-CAGGGACCCGGTATGGGG GGGGCT-3′) and reverse primer HSVgDR (5′-GGCACCA GAGCGTTCTAGTAAAACAAGGGCTGG-3′), for VZV gE, forward primer VZVgEF (5′-CAGGGACCCGGTATG GGGACAGTTAATAAA-3′) and reverse primer VZVgER (5′-GGCACCAGAGCGTTTCACCGGGTCTTATCTAT-3′). To generate vector specific compatible overhangs, the forward and reverse primers were incorporated with victor specific sequence (under lined). Master mix reaction were consisting of 45 µl [5 µl 10 × buffer (50 mM KCl, 1.5 mM $MgCl_2$ and10 mM Tris–HCl; pH 8.3), 1 µl (10 mM) of dNTP, 1 µl of each oligonucleotide primer, 36.5 µl SDW and 0.5 U of AmpliTaq Gold polymerase]. 5 µl of each sample were added including a negative control to a final volume of 50 µl. The cycling programme used included a single initial denaturation at 94 °C for 6 min followed by 40 cycles of denaturation at