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ScienceDirect
Journal of Virological Methods 144 (2007) 17–26
Journal of Virological Methods
www.elsevier.com/locate/jviromet

The attenuation of vaccinia Tian Tan strain by the removal of the viral M1L-K2L genes

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Received 21 July 2006; received in revised form 12 March 2007; accepted 14 March 2007
Available online 24 April 2007

Abstract

To generate a safe vaccinia Tian Tan (VTT)-based vaccine vector, it is necessary to develop a method to attenuate the virus. A modified VTT ($MVTT_{2\text{-}GFP}$) was constructed by replacing the viral M1L-K2L genes with a GFP gene. In comparison to the parental VTT, $MVTT_{2\text{-}GFP}$ lost its replication capacity in rabbit RK13 and human HeLa cell lines. The life cycle of viral replication was blocked at different stages in these two cell lines as determined by electron microscope examination. $MVTT_{2\text{-}GFP}$ was less virulent than VTT for 100-fold by measuring mouse body weight loss after intranasal viral inoculation and for 340-fold by determining the intracranial LD_{50} value in mice. The foreign GFP gene was stable genetically after 10 rounds of passage in Vero cells. Importantly, $MVTT_{2\text{-}GFP}$ elicited both humoral and cell-mediated immune responses to the GFP gene in mice. With two intramuscular inoculations of 10^5 PFU virus, the anti-GFP antibody reciprocal endpoint titer reached over 700 as determined by an ELISA. The number of IFN-γ secreting T cells reached over 350 SFU per million splenocytes against a CD8+ T cell-specific epitope of GFP. Collectively, the removal of the M1L-K2L genes is a useful method to generate an attenuated vaccinia Tian Tan vaccine vector.

Keywords: Vaccinia; Vaccine; Viral vector; Vaccinia Tian Tan; MVTT

1. Introduction

After the declaration of the worldwide eradication of smallpox in 1980, vaccinia virus has been studied for use as a live viral vector for other infectious diseases or cancer therapy (Moss, 1996; Mwau et al., 2004; Paoletti, 1996). In the People's Republic of China, the vaccinia Tian Tan (VTT) strain was used historically as a smallpox vaccine for millions of people, yet its potential as a vaccine vector has not been explored carefully. The full-length sequence of the VTT genome was determined by Jin et al. (1997). Based on sequence comparison, VTT displays genetic features distinct from other vaccinia viruses including the vaccinia western reserve (WR) strain (Hou et al., 1985; Jin et al., 1997; Tsao et al., 1986). In particular, unique deletions or insertions, which affect multiple genes, were identified in the restriction enzyme *Hind*III-C, B and A fragments. These data infer the plausibly distinct biological properties of VTT (Jin et al., 1997). Recently, the biological properties of VTT were characterized in terms of its host cell range and growth properties *in vitro* and virulence *in vivo* (Fang et al., 2005). Although VTT is significantly less virulent than the vaccinia WR strain, it remains lethal in mice after intracranial inoculation and causes significant body weight loss after intranasal inoculation (Fang et al., 2005). These properties have limited its use as a vaccine

Abbreviations: VTT, vaccinia Tian Tan; MVTT, modified vaccinia Tian Tan; MVA, modified vaccinia Ankara; GFP, green fluorescent protein; AIDS, acquired immunodeficiency syndrome; HIV-1, human immunodeficiency virus type one; SIV, simian immunodeficiency virus; SHIV, simian/human immunodeficiency chimeric virus; CTL, cytotoxic T lymphocytes; MOI, multiplicity of infection; PFU, plaque forming unit; p.i., post-infection; EM, electron microscope

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doi:10.1016/j.jviromet.2007.03.012

vector for human use, especially for immune-suppressed individuals. Therefore, further attenuation of VTT is necessary for the development of a useful vaccine vector.

Various viral vector systems have been evaluated for vaccine development (Moss et al., 1996; Santra et al., 2005; Tartaglia et al., 1992). The poxvirus vector is the live recombinant vector which has been studied most intensively. The widely studied vaccinia-based vector is probably the modified vaccinia virus Ankara (MVA). This vector is safe in humans, even in some immunocompromised individuals (Cosma et al., 2003). Moreover, MVA-based vaccines are effective for inducing protective responses against different viruses, such as severe acute respiratory syndrome coronavirus, influenza and respiratory syncytial virus (Bisht et al., 2004; Chen et al., 2005; De Waal et al., 2004; Degano et al., 1999; Olszewska et al., 2004; Sutter et al., 1994; Wyatt et al., 1999). MVA-based vaccines are also effective in delaying the development of AIDS and the progression of the disease in rhesus monkeys infected with simian immunodeficiency virus (SIV) or simian/human immunodeficiency virus (SHIV) (Barouch et al., 2001). However, the immunogenicity profile of MVA as a vaccine vector for HIV-1 is unsatisfactory in humans. A recent phase one trial indicated that an MVA-based HIV-1 vaccine is not very immunogenic in humans (Goonetilleke et al., 2006). It is, therefore, desirable to explore the potential of other vaccinia-based vector systems for stimulating stronger host immune responses in humans.

In this study, a method to generate a modified VTT ($MVTT_{2\text{-}GFP}$) is described. This method involves the genetic modification of the parental VTT genome by deleting the M1L-K2L genes including one host range gene, K1L. The rationale for choosing these genes for deletion corresponds to the lost genes for the deletion II region of MVA genome (Antoine et al., 1998; Meyer et al., 1991). Before this study, it was unknown to what extent a vaccinia virus could be attenuated just by removing the M1L-K2L genes. As the deletion II region of MVA serves as an ideal insertion site for foreign genes, the effectiveness of the corresponding region in the VTT genome for the expression of a foreign gene was also unknown. For these reasons, the phenotypic changes of $MVTT_{2\text{-}GFP}$ in comparison to the parental VTT both *in vitro* and *in vivo*, and the potential use of $MVTT_{2\text{-}GFP}$ as a live viral vector for vaccine development were examined in this study.

2. Materials and methods

2.1. Cell lines and viruses

Thirteen cell lines of various origins were maintained under growth conditions suggested by the American Type Culture Collection (ATCC, Rockville, MD). These cell lines include COS-7, WISH, BHK-21, MRC-5, CHO-K1, HeLa, 293T, RK(15), MDCK, RK13, C6, Vero and NIH/3T3. Primary chicken embryo fibroblasts (CEF) were prepared from 10-day-old embryos. The parental vaccinia Tian Tan 761 strain was obtained from the Institute of Virology at the Chinese Center for Disease Control and Prevention. This VTT 761 strain was derived originally from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). Virus stocks were propagated in Vero cells and then purified by centrifugation through a 36% sucrose cushion. The virus stocks were titrated by a plaque forming assay using crystal violet staining.

2.2. Construction of shuttle vector and $MVTT_{2\text{-}GFP}$

The shuttle vector was constructed by inserting two genome fragments flanking the deletion region of VTT. The GFP gene was placed under a synthetic early/late promoter P_{syn} (Chakrabarti et al., 1997; Wyatt et al., 1996). The $MVTT_{2\text{-}GFP}$ virus was made by a homologous recombination method as described previously (Sutter and Moss, 1992; Sutter et al., 1994; Wyatt et al., 1996). The plaques that express GFP were selected and purified subsequently through six rounds of plaque purification under agarose. The insert in the recombinant virus was determined by a nested PCR using primer pairs flanking the deletion region. PCR primers used for this test were: 5′-GTTTATACAATCCATGCTACTACCTTCGGG-3′ (sense) and 5′-GAATTTCCATTACATCAGGCAGCCACATTGGAAG-3′ (antisense) for the first round reaction and 5′-GGTTCATTG-TTATCCATTGCAGAGGACG-3′ (sense) and 5′-GTAAAGA-TTTTGCTATTCAGTGGACTGGATG-3′ (antisense) for the second round of reaction. The amplification cycles are 95 °C for 2 min followed by 35 cycles of 94 °C for 20 s, 52 °C for 45 s and 72 °C for 3 min plus the last extension of 72 °C for 8 min. Amplified PCR products were purified using a QIAquick PCR purification kit (QIAgen, Valencia, CA, USA) and were subjected to DNA sequencing directly by an automated ABI 377 DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). For comparison, a recombinant VTT_{GFP} was generated by placing GFP gene in a location upstream of the hemagglutinin gene without artificial interruption of the M1L-K2L genes.

2.3. Host cell range, growth property and virulence of $MVTT_{2\text{-}GFP}$

Assays for the determination of viral host cell range, growth property *in vitro* and virulence of $MVTT_{2\text{-}GFP}$ *in vivo* have been described previously (Carroll and Moss, 1997; Fang et al., 2005).

2.4. Electron microscopic (EM) testing

Confluent cell monolayers were infected with 5 MOI of MVTT2-GFP or VTT. The virus was allowed to attach to the cells for 90 min at 37 °C. The cells were then washed with medium three times and incubated at 37 °C for an additional 16 h. The infected cells were then trypsinized to detach them from the culture plates and washed with PBS twice. The cell pellets were fixed in 2.5% glutaraldehyde and processed for examination using a transmission electron microscopy by a routine technique described previously (Wolffe et al., 1996).