Site directed mutagenesis of the carboxyl terminus of human cytomegalovirus glycoprotein B leads to attenuation of viral growth in cell culture

Brief Report


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Summary. A viable human cytomegalovirus (HCMV) mutant was generated harbouring a glycoprotein B (gB) in which the carboxyl-terminal amino acids DRLRHR (aa 885–900) were changed to AALREE. Characterization of the phenotype of the recombinant virus revealed significant reduction of infectious progeny release and only moderate reduction of viral DNA replication indicating its diminished specific infectivity. This observation was in line with immunogold labeling of extracellular virions demonstrating that the amount of gB protein was markedly reduced in the envelope of the mutant virus. Our results suggest that the conserved carboxyl-terminus of the gB molecule is critical for HCMV maturation.

Glycoprotein B (gB) is the major envelope glycoprotein of human cytomegalovirus (HCMV), a beta-herpesvirus. It consists of 906 amino acids (aa) and plays a role in viral attachment, entry, cell-to-cell spread, formation of syncytia in infected cells and represents a prominent target for neutralizing antibodies [2]. The cytoplasmic tail (CT; aa 772–906) of this type 1 transmembrane glycoprotein exceeds with 135 aa the respective length of gBs of other herpesviruses. Various crucial functions have been designated to that portion of the molecule. Following solitary expression, the gB CT has been shown to associate into tetramers [12]. It contains
a number of potential motifs needed for internalization from the surface of infected cells in order to participate in capsid envelopment [11]. Phosphorylation of a serine residue at the extreme carboxyl-terminus (aa 900) appears to play a role in the internalization process [5]. The carboxyl-terminus of gB also contains an aa signal (DRLRHR) that has recently been described as a motif both necessary and sufficient to target transiently expressed gB or a reporter protein to the inner nuclear membrane compartment (INM) [8]. Subsequent studies could further narrow down the signal to the consensus sequence RXR [9].

Previously we reported that mutation of this localization signal is apparently compatible with viral growth [14]. As an extension of this work we report the construction and partial characterization of a viable recombinant HCMV carrying four aa substitutions in the signal motif. The virus constructed should therefore help to both elicit functioning of this carboxyl-terminal gB motif in the context of a viral infection and to further investigate the impact of the alteration in the CT of the multifunctional gB protein on other processes during viral multiplication.

A recombinant HCMV bacterial artificial chromosome (BAC) was constructed in which the aa signal DRLRHR was changed to AALREE (BACsINM; Fig. 1A and B). Transfection of the plasmid BACsINM into primary human fibroblasts was performed as described previously [14] and lead to reconstitution of recombinant virus, designated HCMVsINM. The genome of the recombinant virus was characterized by restriction analysis (Fig. 1B). During cultivation in permissive human fibroblasts the mutant virus HCMVsINM formed small plaques that spread significantly slower compared to the parental BAC-derived wild type virus RV-HB5 [1]. To compare growth kinetics [14] of HCMVsINM with that of RV-HB5 in more detail, fibroblasts were infected at an identical low moi and released infectious virus was titrated for an extended time interval to cover several replication cycles. This analysis revealed that the increase of mutant virus release occurred more slowly and that the total amount of released infectious viral progeny was reduced by approximately two to three orders of magnitude (Fig. 2A). By cotransfection of BACsINM DNA and cos 1029 [6], a cosmid carrying part of the HCMV genome including the gB gene, a revertant virus (R-HCMVsINM) was rescued whose growth behavior was identical to that of wild type virus (Fig. 2A).

To elucidate the growth impairment of the mutant virus more closely, intra- and extracellular viral DNA was quantified in an identical experiment. For the amount of intracellular viral DNA of HCMVsINM infected cultures only a moderate decrease was observed compared with RV-HB5- or R-HCMVsINM infected cultures (Fig. 2B). No accumulation of viral DNA in HCMVsINM infected cells occurred, indicating that assembly and egress of mature particles was not impaired. These findings were supported by separate electron microscopic studies that revealed no apparent differences between mutant and wild type virus infected cells regarding numbers of viral capsids in the nucleus and the cytoplasm (data not shown). Likewise, quantities of extracellular viral genome equivalents in the medium of HCMVsINM infected cultures were again reduced only by about half to one order of magnitude when compared to RV-HB5- and R-HCMVsINM infected cultures