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Enhancement of safety and immunogenicity of the Chinese Hu191 measles virus vaccine by alteration of the S-adenosylmethionine (SAM) binding site in the large polymerase protein

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ABSTRACT

The live-attenuated measles virus (MV) vaccine based on the Hu191 strain has played a significant role in controlling measles in China. However, it has considerable adverse effects that may cause public health burden. We hypothesize that the safety and efficacy of MV vaccine can be improved by altering the S-adenosylmethionine (SAM) binding site in the conserved region VI of the large polymerase protein. To test this hypothesis, we established an efficient reverse genetics system for the rMV-Hu191 strain and generated two recombinant MV-Hu191 carrying mutations in the SAM binding site. These two mutants grew to high titer in Vero cells, were genetically stable, and were significantly more attenuated *in vitro* and *in vivo* compared to the parental rMV-Hu191 vaccine strain. Importantly, both MV-Hu191 mutants triggered a higher neutralizing antibody than rMV-Hu191 vaccine and provided complete protection against MV challenge. These results demonstrate its potential for an improved MV vaccine candidate.

1. Introduction

Measles virus (MV) is an enveloped virus with a non-segmented, negative-sense (NNS) RNA genome in the family *Paramyxoviridae*, order *Mononegavirales* (Radecke et al., 1995). In developing countries, measles is still a leading cause of mortality in children (Griffin and Oldstone, 2009; Tangy and Naim, 2005), though vaccination is an effective, economical, and safe way to prevent outbreaks (Bester, 2016; de Vries et al., 2008). In early 1960, a live-attenuated vaccine based on the Hu191 strain of MV was developed and is currently widely used for immunization in all provinces of China (Zhang et al., 2009). While this vaccine is efficacious, it has associated adverse effects. Many vaccinated infants and children in China experienced side effects ranging from skin rashes, itching, swelling, and to high fever (Bester, 2016; Shu et al., 2011). Additionally, outbreaks of measles have been increasing significantly in the past a few years in China, particularly the increasing proportion of adult and infant cases (Ma et al., 2016; Zhang et al., 2016). The infected adults had received measles vaccination during childhood; still remain susceptible to infection with the measles virus, as the population immunity against measles after vaccination gradually reduces with time (Abad and Safdar, 2015; Gao et al., 2017; Ma et al., 2016; Zhang et al., 2016). Thus, there is an increasing urgency to develop a safer, more efficient MV vaccine for eradication of measles in China.

Reverse genetics system has been established for many NNS RNA viruses including the vesiculovirus, morbillivirus, respirovirus, and pneumovirus (Neumann et al., 2002). Similar to other NNS RNA viruses, the minimal machinery for MV transcription and replication is the ribonucleoprotein (RNP) complex, which consists of the nucleocapsid (N)-RNA template tightly associated with the RNA-dependent RNA polymerase, the large (L) protein and the phosphoprotein (P). Assembly of replication-competent RNPs is essential to the rescue of NNS RNA viruses (Bukreyev et al., 1996; Clarke et al., 2000; Garcin et al., 1995; Gassen et al., 2000; Jin et al., 1998; Lawson et al., 1995).

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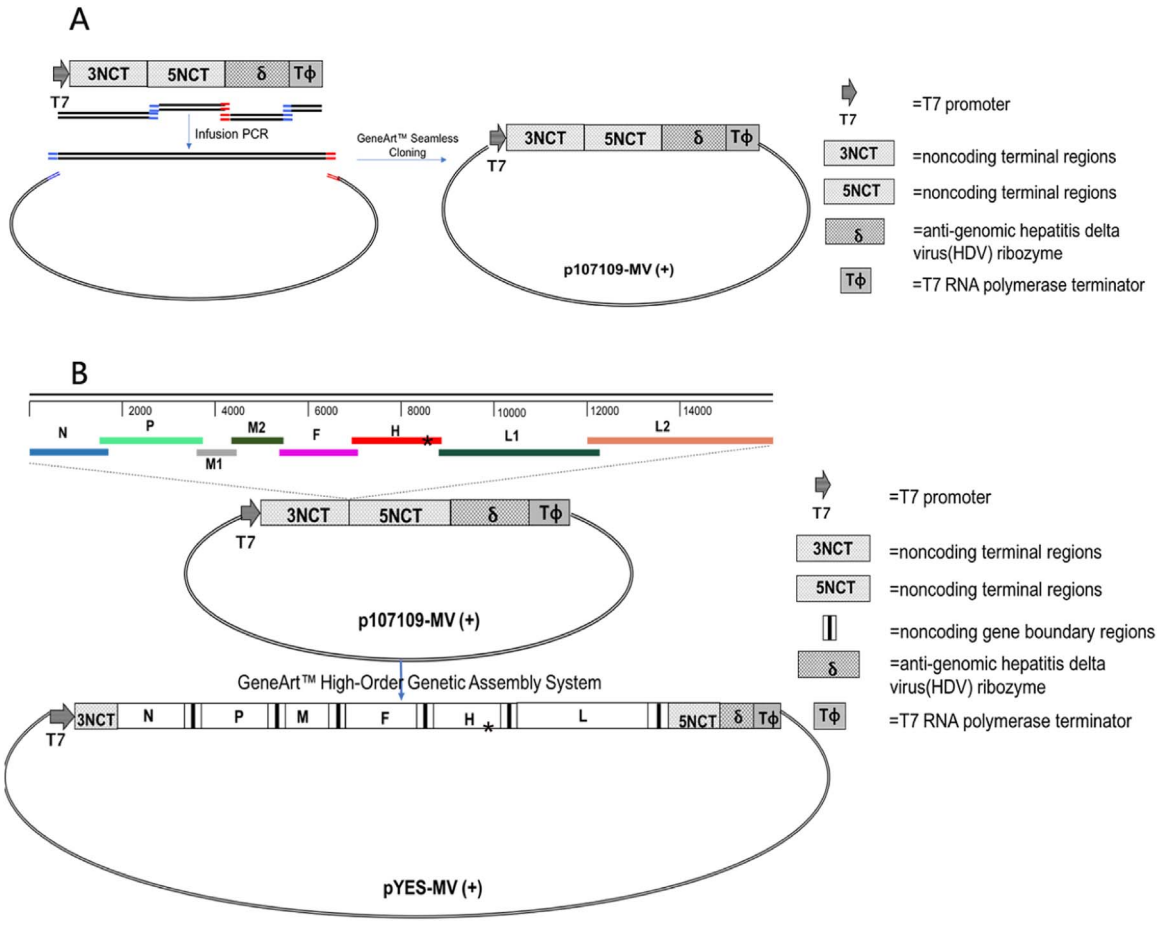

Fig. 1. Construction of a full-length cDNA clone for MV-Hu191. The T7 promoter, 3′ and 5′ non-coding termini (NCT), antigenomic HDV ribozyme and T7 terminator were assembled in several rounds of fusion PCR, and inserted into pYES-2 using a "seamless" cloning strategy, resulted in the construction of p107109-MV(+) (A). Eight overlapping fragments containing the full-length MV genome were assembled into p107109-MV(+), creating pYES-MV(+) (B). A spontaneous mutation (C to U) in the H gene that distinguishes the lab-propagated parental virus and rescued recombinant virus was marked by "*" (B).

This can be achieved by co-transfection of a plasmid encoding a full-length antigenomic cDNA together with plasmids encoding N, P, and L genes. Previously, several groups have already successfully rescued infectious MV from cDNA clones (Duprex et al., 1999; Kovacs et al., 2003; Nakatsu et al., 2006; Parks et al., 1999; Radecke et al., 1995; Sidhu et al., 1995). The reverse genetics system can facilitate the rational design of safer, more efficient measles vaccine candidates.

The L protein of NNS RNA viruses possesses the majority of enzymatic activities for transcription and replication (Ferron et al., 2002; Poch et al., 1990; Whelan et al., 2004). During transcription, NNS RNA viruses synthesize mRNAs that are capped and methylated at the 5'end and polyadenylated at the 3' end. Recent studies have shown that the entire mRNA capping and methylation machinery of NNS RNA viruses is distinct from their host (Ferron et al., 2002; Furuichi and Shatkin, 2000; Ma et al., 2014; Ogino and Banerjee, 2007; Zhang et al., 2014). Using vesicular stomatitis virus (VSV) as a model, it was found that VSV mRNA capping is catalyzed by an RNA:GDP polyribonucleotidyltransferase (PRNTase) in the L protein that transfers a monophosphate RNA onto a GDP acceptor (Li et al., 2008; Ogino and Banerjee, 2007). The mRNA cap methylation in NNS RNA viruses is also unusual in that a single region in the L protein catalyzes both guanine-N-7 (G-N-7) and ribose 2′-O (2′-O) methylation (Li et al., 2006; Rahmeh et al., 2009). Thus, mRNA cap formation is an excellent target for development of antiviral drugs and live vaccine candidates for NNS RNA viruses. Based on the sequence alignments, the L protein contains six conserved regions (CR) numbered I to VI. Recent studies showed that CR V of the L protein possesses an mRNA capping enzyme whereas CR VI is responsible for mRNA cap methyltransferase (MTase) activity (Li et al., 2008; Ogino et al., 2005). It was shown that mutations to the capping enzyme were lethal to the virus. However, mutations to MTase region yielded recombinant viruses that were attenuated *in vitro* and *in vivo*. This suggests mRNA cap MTase is a novel target for rational design of live attenuated vaccines for NNS RNA viruses. This novel concept has recently been tested in several NNS RNA viruses including VSV, avian metapneumovirus (aMPV), human metapneumovirus (hMPV), and rabies virus (RABV) (Li et al., 2006; Ma et al., 2014; Sun et al., 2014; Tian et al., 2015; Zhang et al., 2014). It was shown that recombinant viruses lacking MTase activity are highly attenuated *in vitro* and *in vivo,* yet retain optimal immunogenicity.

We hypothesized that engineering mutations to the MTase region of MV L protein would lead to further attenuation of the current live attenuated vaccine strain, enhancing the safety of MV vaccine. To test this hypothesis, we established a robust reverse genetics system based on a Chinese MV vaccine strain MV-Hu-191, allowing us to recover recombinant MV in BHK cells stably expressing T7 RNA polymerase (Xu et al., 2011; Zhang et al., 2014). Subsequently, two recombinant MVs with amino acid (aa) substitutions in the S-adenosylmethionine (SAM) binding site of L protein (rMV-Hu191-G1788A and rMV-Hu191-G1792A) were successfully recovered. These two MTase-defective mutants had delayed replication kinetics, grew to high titers, and were genetically stable through 15 passages in cell culture. Both MV mutants were significantly more attenuated *in vitro* and *in vivo* compared to the parental vaccine strain. Interestingly, both mutants induced significantly higher neutralizing antibody titers compared to the parental